

Supplemental Material

Table S1. ChIP-Seq read statistics

Sample ID	Total reads	Mapped reads	Alignment percentage
17 AAR H3K9Me2	46,464,760	43,314,561	93.22%
17 RM H3K9Me2	42,529,152	36,486,851	85.79%
19 AAR H3K9Me2	46,432,758	41,453,073	89.28%
19 RM H3K9Me2	47,421,330	39,779,990	83.89%
22 AAR H3K9Me2	27,843,170	25,093,645	90.12%
22 RM H3K9Me2	30,375,422	26,930,741	88.66%
23 AAR H3K9Me2	55,683,060	48,354,090	86.84%
23 RM H3K9Me2	53,956,742	48,575,121	90.03%
27 AAR H3K9Me2	56,970,418	50,556,075	88.74%
27 RM H3K9Me2	46,226,168	42,043,416	90.95%
30 AAR H3K9Me2	37,993,234	33,554,321	88.32%
30 RM H3K9Me2	54,274,792	48,180,580	88.77%
17 AAR Input	54,830,472	51,482,772	93.89%
17 RM Input	53,865,358	46,884,016	87.04%
19 AAR Input	59,934,412	53,550,931	89.35%
19 RM Input	59,816,972	53,550,004	89.52%
22 AAR Input	56,815,734	52,596,352	92.57%
22 RM Input	71,595,832	65,616,757	91.65%
23 AAR Input	71,275,420	62,669,043	87.93%
23 RM Input	71,239,678	64,876,583	91.07%
27 AAR Input	61,597,486	55,704,829	90.43%
27 RM Input	52,275,432	49,100,216	93.93%
30 AAR Input	59,142,706	54,035,048	91.36%
30 RM Input	57,672,304	53,114,715	92.10%

Table S2. List of significantly downregulated genes in AAR enriched in H3K9me2.

Gene symbol	p-value	Fold change (downregulated)
2310067B10Rik	0,002480138	102,1654158
2810006K23Rik	0,00162463	10,57328448
3110002H16Rik	0,001412289	13,20571078
Aagab	0,001634436	16,78780261
Abce1	0,000169836	21,58335747
Acad11	0,000771312	48,93304818
Acadm	0,00012548	14,58513162
Acbd3	0,002421549	19,62370165
Acs1	0,001771233	28,41238432
Acss2	0,000169588	34,7633871
Actn2	0,000434284	40,40323063
Actn4	0,000465074	10,14473799
Adck3	0,001925546	12,84473481
Adipor1	0,000186977	12,20307223
Afg3l2	6,39407E-05	10,54513383
Agk	0,000655525	13,70673736
Aldh4a1	0,000356014	16,59000252
Aldh6a1	0,00047691	11,37147505
Ank	0,000332791	14,38589675
Ankrd46	0,003093555	11,58600068
Apbb2	0,003125797	20,78966059
Aplp2	0,000122655	81,78046438
App	0,000243482	19,56518369
Arfp1	0,000851222	21,22537665
Arhgap5	0,001418133	11,93718286
Asb8	0,001300531	18,96999311
Ascc3	0,000241057	12,84401188
Asph	0,00397376	27,0292898
Atp1a1	0,000213355	18,81625224
Atp6v0a1	0,001555441	12,75132844
Azin1	0,010354842	11,0444908
Bcam	0,00049915	29,83549277
Birc6	0,014263687	10,06915002
Bmpr1a	0,001808977	12,23449629
Cab39	0,003642909	13,12810793
Calm3	0,001242676	235,197954
Camta1	0,002100626	11,39364851
Caprin1	0,001322025	12,29649847
Cat	0,000254316	84,60125736
Cbfb	0,000508865	12,57479957
Cct6a	0,001527757	42,11884589

Cd164	0,001167193	11,34085299
Cdh2	0,000908897	17,72072593
Cdkn2c	0,000225008	12,2466754
Cecr5	0,00014144	20,72752709
Celf2	3,98137E-05	18,90688779
Cfl2	0,000151221	10,96141013
Cmya5	0,001165487	67,06427289
Cnst	0,000124327	11,74842902
Cog6	0,000172356	37,40364985
Coro6	0,000733336	11,02687052
Crat	0,001932849	56,28154782
Crhr2	0,000201616	13,19971935
Ctbp2	0,00599797	13,88894729
Ctnna1	0,001326885	5,216371792
Ctps	0,002562779	12,75537757
Cttn	0,005662566	10,81261671
Cul4a	0,006352797	24,7262397
Decr2	0,004211541	13,92199445
Dek	0,000412358	43,1205926
Dennd5a	0,000721373	22,80127905
Dhx32	0,003290187	19,54859003
Dhx40	0,000391409	10,64299511
Dlg1	0,0006463	18,39358257
Dpf2	0,000356996	21,300258
E2f6	4,55846E-06	42,75022921
Ece1	0,006211245	10,42712303
Eef2	1,82376E-05	85,87443221
Ehd4	0,001479994	46,15259939
Eif3c	0,002223805	24,96775585
Eif4g2	0,000263924	44,47364205
Eif4h	0,002347282	20,9419532
Eif5	4,85775E-05	18,24890444
Eif5a	0,000122737	68,24691843
Elp3	0,001337693	11,6096156
Epc2	0,000995765	12,76577305
Eral1	0,000478964	18,39878317
Etf1	2,94653E-05	11,46065663
Exoc7	0,000472115	13,67825182
Fam134b	0,001951167	59,87939957
Fam20b	0,00095375	10,82778507
Fbxo32	0,004956845	14,83783657
Fbxw4	0,001641741	10,34838694
Fcho2	0,01304067	13,56650785
Flot2	0,001559024	22,85469245
Gab1	0,000745502	10,88356277

Gapvd1	0,001477674	23,72063704
Gas6	0,000788084	11,26188368
Gbas	0,000216482	33,96662569
Gbf1	0,004217147	12,49705716
Gja1	0,004757617	13,58229154
Gmcl1	0,000104169	11,7866996
Gna12	3,14655E-05	67,57961376
Gns	0,005321713	27,16935761
Golga4	7,35722E-05	34,21677775
Gpam	0,000744526	22,63013052
Gpr116	0,000140207	10,30373507
Grk5	0,000201126	13,0766596
Gsn	0,000444621	26,83864628
Gspt1	0,003247701	33,94253002
Gtf3c1	0,027046379	10,18488757
H2afy	0,000400251	10,550784
Hba-a2	2,57738E-05	12,97466154
Hbs1l	0,000181824	15,18860062
Hdgf	5,63644E-05	20,68218566
Hdgfrp2	0,005069079	19,16121448
Hdlbp	0,000105784	194,8942139
Hnrnp1	0,000227103	36,48405548
Hnrnp1	0,00021706	20,68093418
Hspa9	0,00035797	10,49925459
Il10rb	8,109E-05	16,27563019
Impa1	0,002341459	11,73366253
Ipo5	0,003425824	11,81630856
Itgb1	0,000462383	87,94747787
Ivd	0,000153865	10,52502205
Kcnp2	0,000853735	13,32989028
Kctd9	0,001566204	27,55694877
Kdm1b	0,002731403	10,61342139
Kif16b	0,000255956	35,45975099
Kpna1	0,008243067	17,84950734
Lemd3	0,000150823	13,09381867
Lhfp	0,000233585	23,74089058
Limd1	0,000614375	12,65934188
Lrpprc	0,002478513	22,63791133
Lrrc2	0,001084912	12,04946135
Lrrfip2	0,009649554	10,36548093
Man1c1	0,001099435	17,07991452
Mcf2d	0,000253562	15,87184735
Meis2	0,000710042	7,514417114
Mfn2	0,000341302	16,07946834
Mknk2	0,004451539	14,92024841

Mtor	0,001913992	6,865978311
Mybpc3	4,46256E-05	100,8929879
Nap114	0,00107747	14,04586433
Nbr1	0,00051085	29,14596977
Ndrq2	1,4613E-05	29,05827184
Nedd4	1,00138E-05	14,82787034
Nek9	0,00077353	12,05029951
Nfe2l2	0,00051056	11,13959479
Nid1	4,28698E-05	15,986655
Nop2	0,001075354	10,75355074
Nploc4	0,019859953	10,13963735
Npr2	0,007077947	12,67156623
Nqo1	0,003312753	13,3287631
Nup93	0,001277829	10,2546749
Ogdh	0,000624526	50,11253448
Osbpl9	8,52387E-05	41,30718237
Ostm1	0,00755069	12,47577989
P4ha1	0,000307651	49,63564552
Pabpc1	0,000113612	23,33800038
Pcnt	0,000757784	16,00057893
Pdcd7	0,002459516	12,89053065
Pde4a	0,000632228	10,15468231
Pecam1	0,00063725	10,10208373
Pfn2	0,000259025	10,29655272
Phtf2	0,001267849	16,87241698
Pip5k1b	0,00088603	11,30926567
Pitpnb	0,000465497	11,0318883
Plekha3	0,000175749	10,65349429
Plin3	0,000632742	23,80524381
Plk2	0,000651124	10,6472238
Pnrc2	8,07038E-05	36,87491963
Poglut1	0,002045177	14,93528539
Ppig	1,39452E-05	15,80543514
Ppil1	0,000120386	26,71874982
Ppp1r8	0,000212018	11,58699012
Ppp2r1a	0,000411018	32,52013858
Ppp3r1	0,001031523	22,15290128
Ppp6r3	0,000754381	13,53915551
Prkaa2	0,00202781	16,87456609
Prkab1	0,00010437	11,45132791
Prkaca	0,000175228	26,14050034
Psap	9,04259E-05	15,73310482
Ptbp2	0,000788301	10,8791403
Ptp4a3	0,000457989	10,86221297
Ptplad1	3,00574E-05	12,08623087

Ptpn12	0,00128104	12,68952974
Ptpra	0,000594321	10,03728479
Pygb	0,000272386	20,0571157
Qk	5,49823E-05	22,16162783
Rab12	0,000225852	73,71341285
Rab18	0,002872983	56,66138259
Rbl2	0,004371049	24,02031807
Rbm22	0,009346897	10,39801798
Rdx	0,000794098	47,34289914
Rftn1	0,002772576	23,64484616
Rpa1	0,000367671	16,56185655
Rragc	0,002021981	57,27965905
Rras2	0,002181742	11,05308969
Rsad2	0,003582919	10,98703917
Scn5a	0,002479289	10,9212872
Serinc3	0,000337664	59,26992801
Sf3a1	0,002727226	12,67797831
Sipa1l2	0,036047307	14,97917487
Slc27a1	0,001305203	76,44728444
Slc2a4	0,000418931	90,96880523
Slc35a5	0,003303153	13,75541966
Slc35f5	0,000251782	134,6780101
Slc41a3	0,001648888	21,03516749
Smad4	0,000916134	18,38573823
Smoc2	0,00095619	16,2858425
Snx27	0,000279081	20,97613979
Sorbs1	0,000362116	37,98839883
Spop	0,007713447	66,32222029
Srl	0,00012279	30,02641854
Srsf1	0,000344176	25,53965267
St3gal5	0,001434082	11,56864908
Stard7	0,000354167	34,83416799
Stat3	9,80807E-05	20,53296265
Stip1	0,001026156	15,51410429
Stk24	0,000249265	47,80400048
Strn3	0,000724033	62,95439524
Stx12	0,000284974	12,09395156
Svil	0,002679208	24,81322241
Tarsl2	0,001153426	10,28001525
Tbk1	0,001644334	14,32187723
Tex2	0,000370307	16,28865245
Tjp1	0,000182526	10,14172285
Tm9sf3	3,06678E-05	23,05582684
Tmem165	0,001539312	12,97909332
Tmem50b	0,001609145	19,31957028

Top1	0,002762039	20,39952706
Tsc22d4	0,002290293	11,27983258
Uba2	0,003556512	10,66045603
Ube2d1	0,000122634	14,924873
Ube2w	0,000213766	16,60721786
Unc45b	0,000245605	40,90894282
Usp4	0,000126441	26,79379444
Vapb	0,003434344	12,98048335
Wdr5	0,002128075	20,24138635
Wsb1	0,000231005	50,16556869
Yars	0,002201753	11,35377538
Ypel5	0,000540469	11,21804795
Ywhae	0,000316369	31,07580804
Zbtb20	0,002213139	11,58264429
Zmym5	0,004863197	10,87247774

Table S3. Pathway analysis of downregulated, H3K9me2-enriched genes.

Pathway Name	No of reference genes in category	Entrez Gene Ids	Expected number of genes in category	Ratio of enrichment	Raw P-values	Adjusted P-values
Insulin signaling pathway	137	108079 53413 20411 19079 17347 14252 110078 18747 12315 20528	0,56	17,97	2,94E-10	2,09E-08
Regulation of actin cytoskeleton	216	60595 227753 16412 66922 18719 19684 14673 12632 18645 11472	0,88	11,4	2,40E-08	8,52E-07
Adipocytokine signaling pathway	68	108079 72674 14081 19079 20848 20528	0,28	21,73	3,73E-07	8,83E-06
Arrhythmogenic right ventricular cardiomyopathy	74	60595 14609 16412 12385 11472 12558	0,3	19,96	6,19E-07	9,53E-06
Adherens junction	75	60595 17128 20411 12385 21872 11472	0,3	19,7	6,71E-07	9,53E-06
Tight junction	137	60595 12385 66922 21872 13043 51792 11472	0,56	12,58	1,64E-06	1,94E-05
Metabolic pathways	1184	18451 75612 14732 20454 14081 12359 11975 51797 230815 104776 18293 18719 55980 11364 69923 212647 60525 56357	4,81	3,74	2,07E-06	2,10E-05
Leukocyte transendothelial migration	120	60595 16412 12385 18613 11855	0,49	12,31	1,05E-05	9,32E-05

		11472				
Ubiquitin mediated proteolysis	140	17999 50995 99375 216080 66799 12211	0,57	10,55	2,51E-05	2E-04
mRNA surveillance pathway	93	225363 56422 18458 14852 51792	0,38	13,24	4,11E-05	3E-04

Figure S1. Levels of H3K4me3 and H3K27me3 are unaltered in cardiac biopsies from IPC and sham animals. Western blot analysis of histone modifications in cardiac biopsies from the area at risk (AAR) and remote myocardium (RM) from animals (n=3) subjected to ischemic preconditioning or sham controls. Two-way ANOVA with Bonferroni post hoc analysis was used to test the effect of treatment but revealed no statistically significant changes.

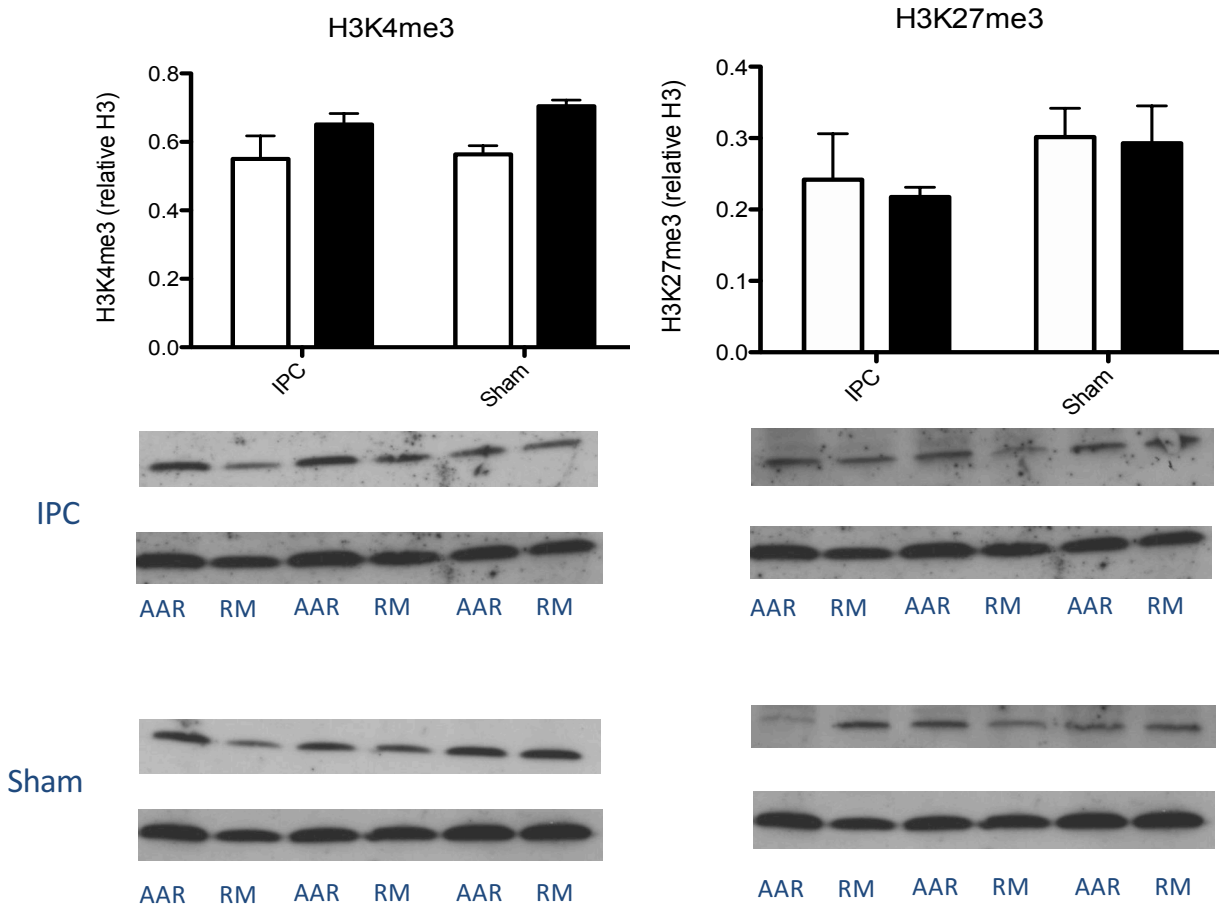


Figure S2. G9a levels are unaltered in cardiac biopsies from IPC mice. Western blot analysis of cardiac biopsies from the area at risk and remote myocardium of IPC mice (n=3) revealed no significant difference in G9a levels in the area at risk.

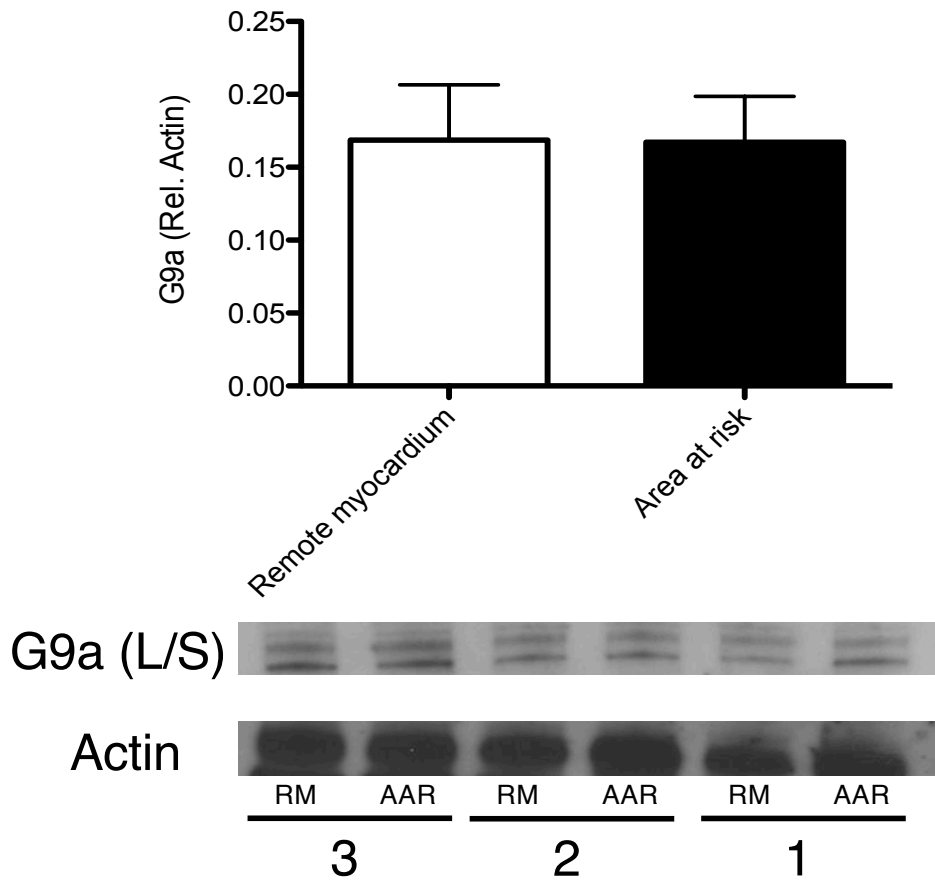


Figure S3. H3K9me2 heat map. Genes that were both enriched in H3K9me2 and transcriptionally repressed were identified (n=236). Differentially enriched peaks within those genes were calculated using EdgeR and a heat map based on the read number for those peaks was generated using MicroScope.

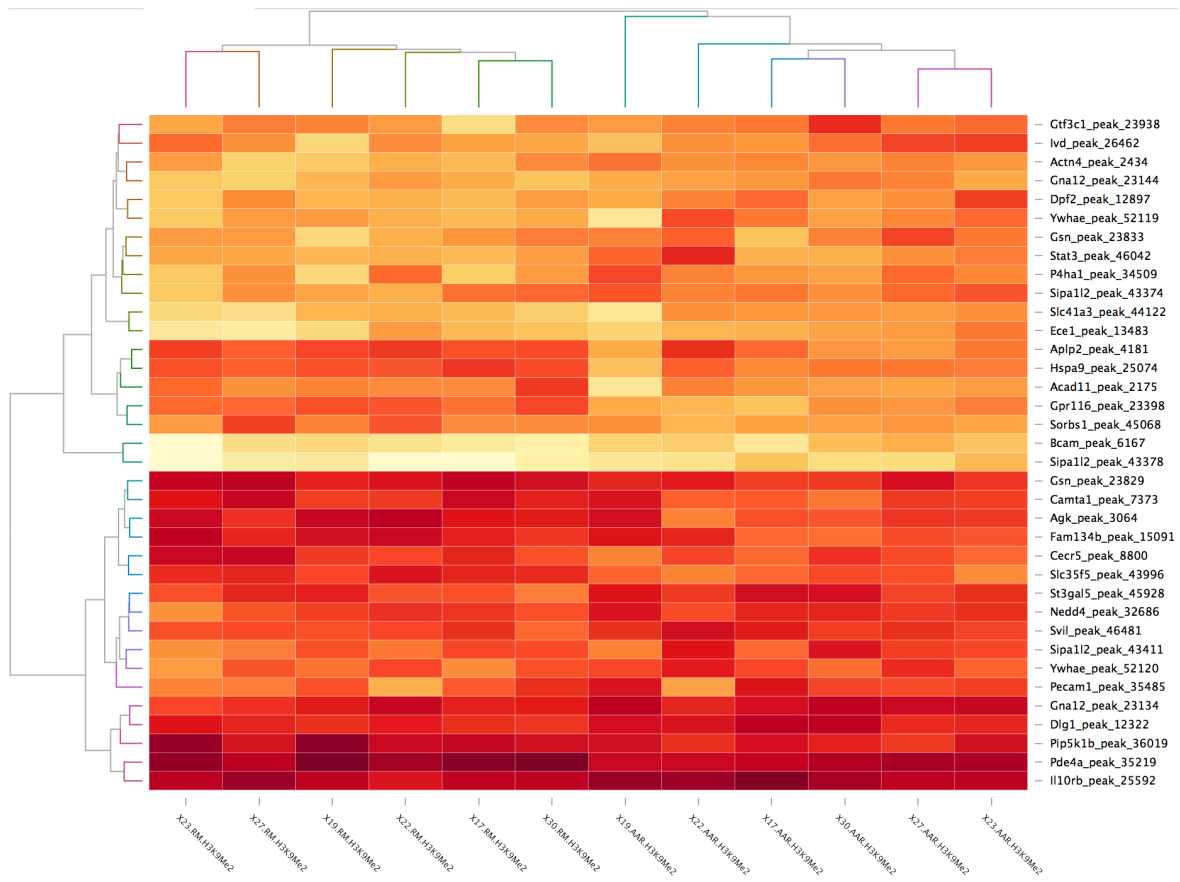


Figure S4. Controls for the H3K9me2 ChIP-PCR. As a control for the H3K9me2 ChIP, enrichment of H3K9me2 at the MyoD promoter and lack of H3K9me2 at the Gapdh promoter was assessed with ChIP-qPCR on cardiac biopsies from sham mice (n=3). As a negative control, an IgG IP was performed on the same samples.

