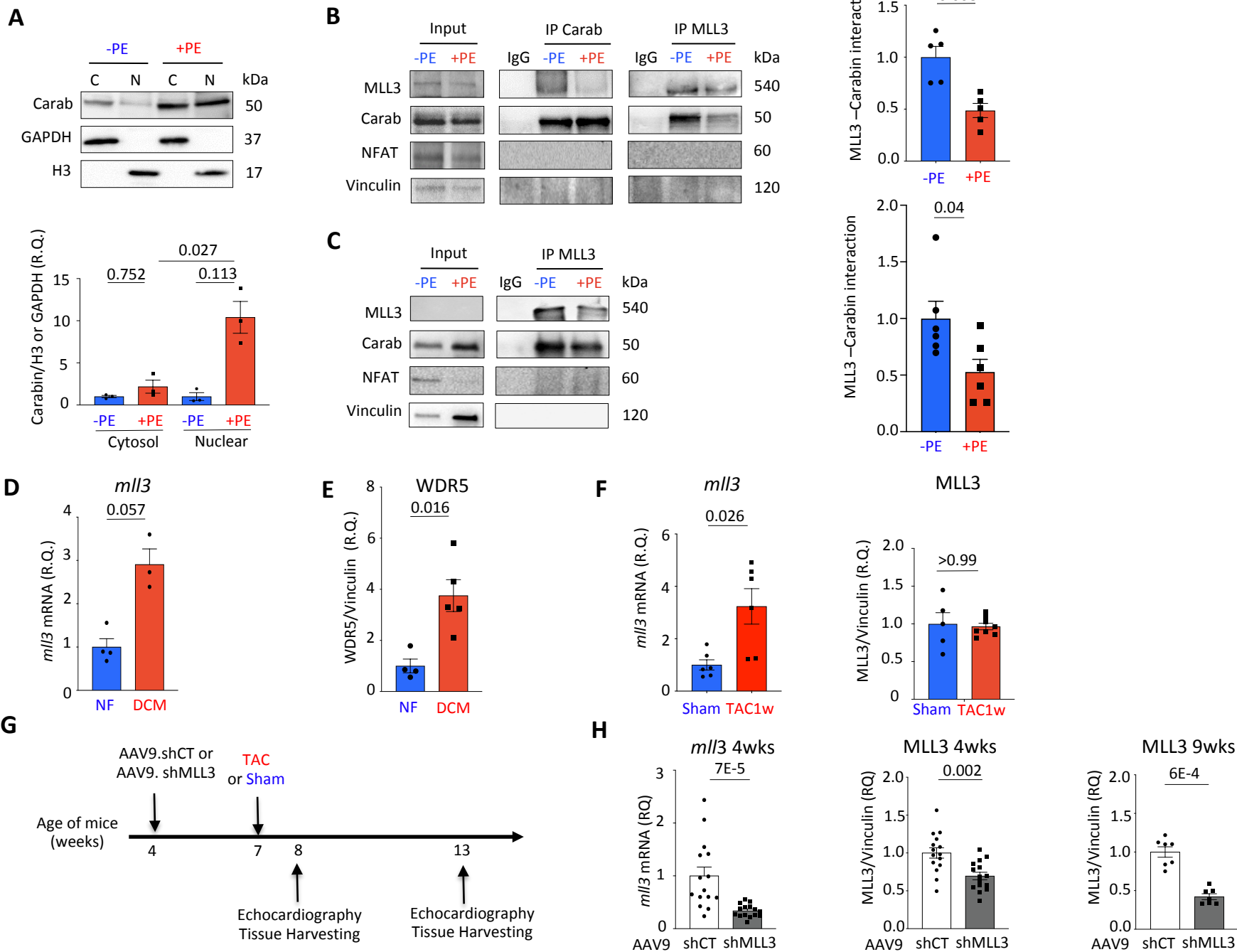


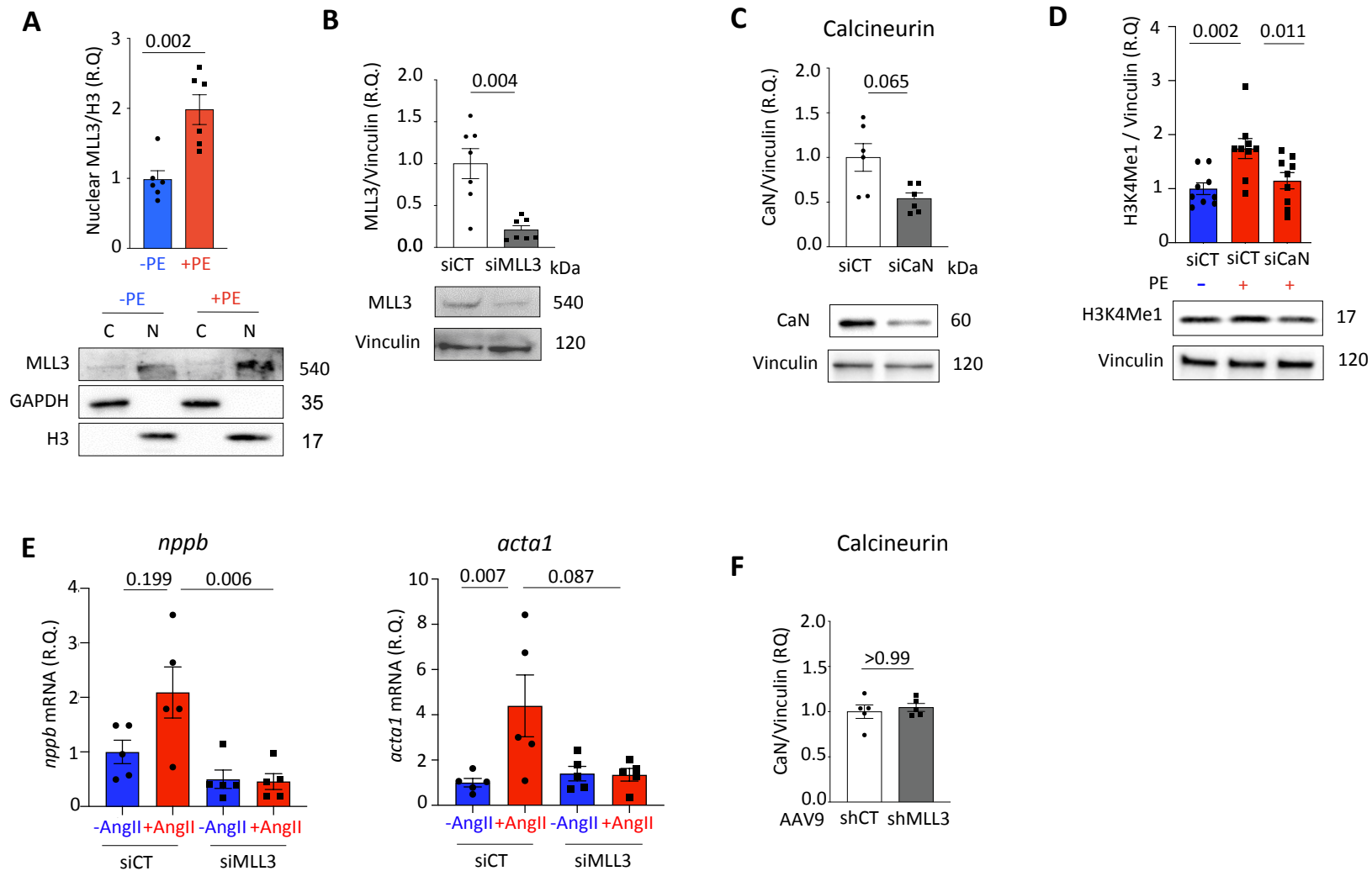
**SUPPLEMENTAL
FIGURES 1-6**

Supplemental Figure 1



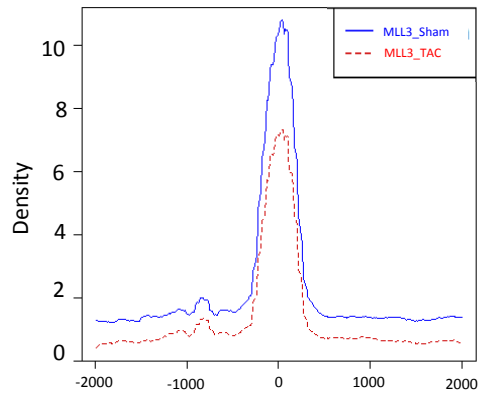
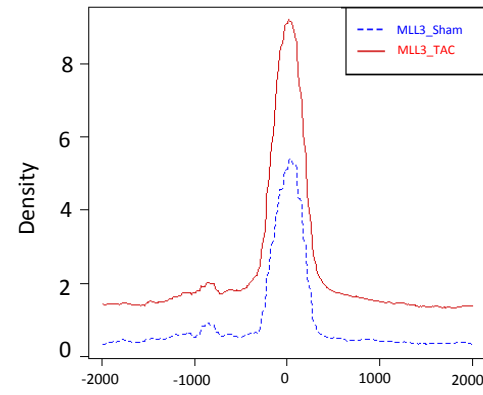
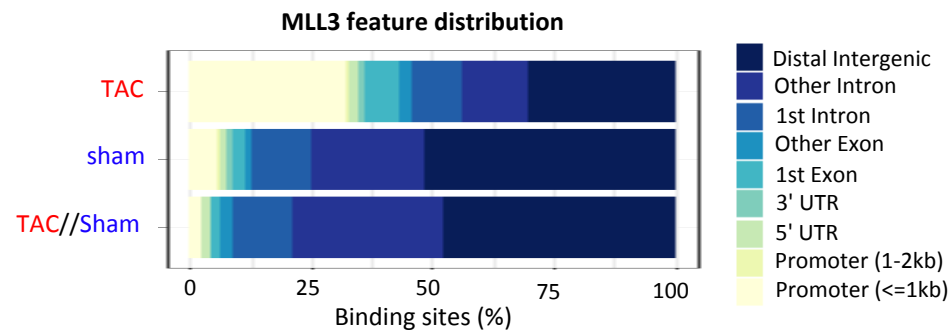
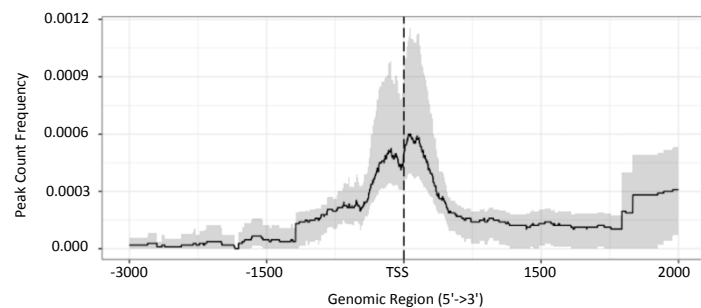
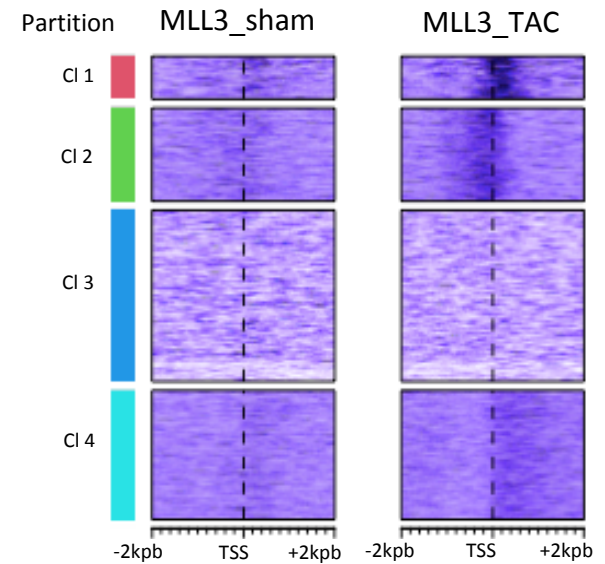
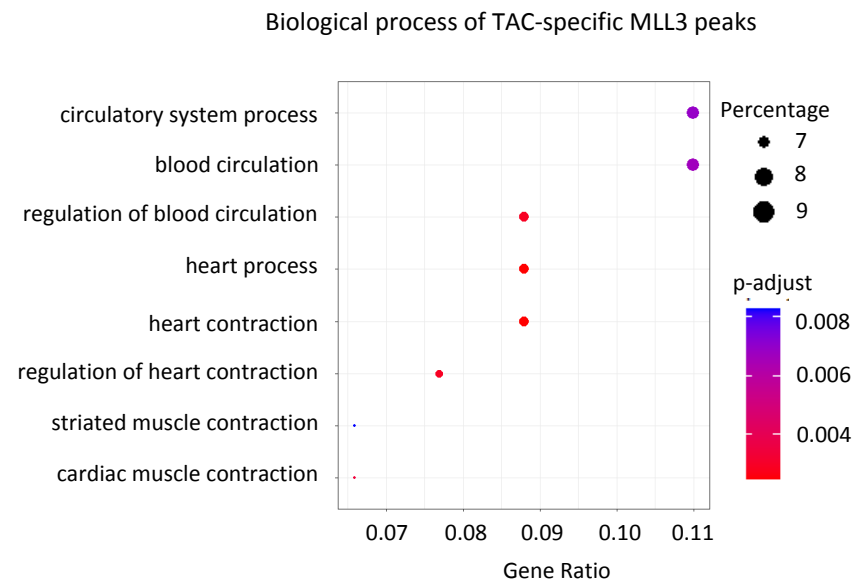
Supplemental Figure 1.

A, Quantification and representative immunoblot of Carabin (Carab) expression in the nuclear and cytoplasmic fractions of NRVM treated (+) or not (-) with PE (10 μ M, 24 h) (n = 3) **B**, Representative immunoblot of immunoprecipitation (IP) showing the interaction of Carabin with MLL3 in NRVM stimulated or not with PE (10 μ M, 30 min) (n=5). Input is a control of cell lysates. Histogram shows the ratio of immunoprecipitated Carabin to immunoprecipitated MLL3. **C**, Representative immunoblot of MLL3 immunoprecipitation experiment showing the interaction of Carabin with MLL3 in isolated adult rat cardiomyocytes treated or not with PE (10 μ M, 30 min). Histograms showing the ratio of immunoprecipitated MLL3 to immunoprecipitated Carabin (n=6). Results are expressed relative to -PE condition (A-C). **D**, Relative mRNA expression of *mll3* in human hearts from patients with dilated cardiomyopathy (DCM, n=5) and non-failing (NF, n=4) heart **E**, Quantification of WDR5 protein expression in left ventricular myocardium from patients with dilated cardiomyopathy (DCM; n=4) or non-failing (NF; n=5) control biopsies. **F**, Relative mRNA and protein expression of MLL3 in mouse hearts from one-week TAC and Sham mice (mRNA n=6 ; protein n=5 and 8 in sham and TAC condition respectively). **G**, Experimental strategy to evaluate the effect of MLL3 knock-down on TAC-induced cardiac hypertrophy. **H**, Quantification of MLL3 mRNA and protein expression in hearts 4 weeks (4wks) or 9 weeks (9wks) after AAV9-shCT (sh-CT) or AAV9-shMLL3 (sh-MLL3) injection (n=15 for 4wks and n=7 for 9wks). Results are expressed relative to non-failing control (D-F, H) and as mean \pm SEM. Differences between groups were analyzed by a Friedman test with a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli (A) or a Mann-Whitney U test (B, C, D, E, F, H). Black lines indicate compared groups with the corresponding p-value. Differences were considered significant when $P < 0.05$.



Supplemental Figure 2.

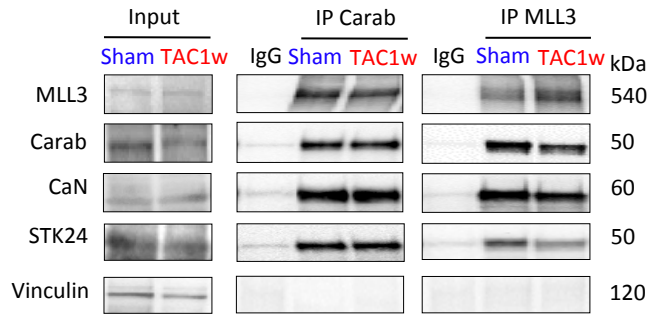
A, Quantification and representative immunoblot of MLL3 expression in the nuclear fraction of NRVM treated or not with PE (10 μ M, 24 h) (n=6). RQ, relative quantity. **B**, Quantification and representative immunoblot of MLL3 expression in NRVMs transfected with MLL3 targeted siRNA (siMLL3) or siRNA control (siCT) (n=7). **C**, Quantification and representative immunoblot of Calcineurin expression in NRVMs transfected with Calcineurin targeted siRNA (siCaN) or siRNA control (siCT) (n=6). **D**, Quantification and representative immunoblot showing the effect of siRNA against Calcineurin (siCaN) on H3K4Me1 level in NRVMs treated or not with PE (10 μ M, 3 h) (n=9). **E**, Relative expression of cardiomyocyte hypertrophy gene markers (*nppb*, *acta1*) in siCT or siMLL3-transfected NRVM treated (+AngII) or not (-AngII) with Angiotensin II (1 μ M, 24 h) (n= 5). **F**, Quantification of Calcineurin protein expression in hearts of control AAV9-ShCT or AAV9-shMLL3 treated mice subjected or not to TAC surgery for 1 week (n=5) Values are normalized to the siCT or shCT (B,C,F) and without PE (D) or Ang (E). Results are expressed as mean \pm SEM. Differences between groups were analyzed by a Mann-Whitney U test (A, B, C, F) or a Kruskal-Wallis test with with two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli (D, E). Black lines indicate compared groups with the corresponding p-value. Differences were considered significant when $P < 0.05$.

A

B

C

D

E

F


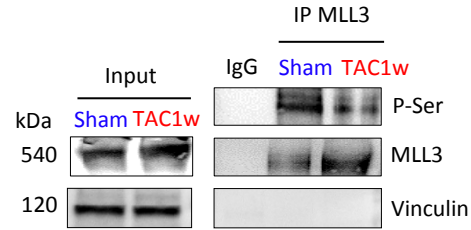
Supplemental Figure 3.

A, Signal intensity plot representing MLL3 ChIP-seq signal in TAC condition at +/- 2 kpb from MLL3 peak centers obtained in Sham. **B**, Signal intensity plot representing MLL3 ChIP-seq signal in Sham condition at +/-2 kpb from MLL3 peak centers obtained in TAC. **C**, Genomic distribution of MLL3 sham-specific and TAC-specific, and sham and TAC co-binding regions. Genomic regions are color coded according to the labels on the right. **D**, ChIP-seq profiles at -3 kpb/+2 kpb to Transcription Start Site (TSS) for TAC-specific MLL3 binding sites **E**, Heatmap of k-means clustered MLL3 ChIP-seq signals in Sham and TAC conditions at +/- 2 kpb to TSS. **F**, Gene Ontology analysis showing the biological processes associated with the genes located closest to TAC-specific MLL3 peaks.

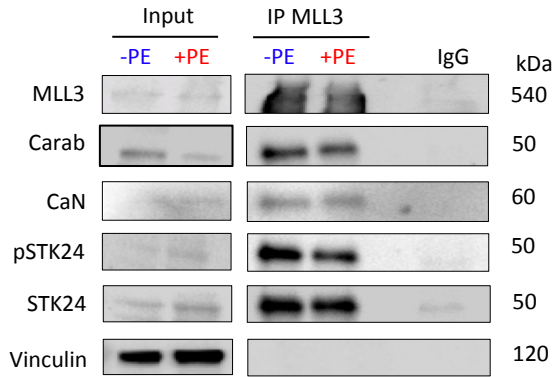
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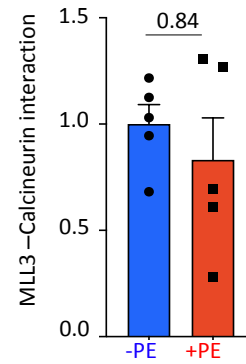
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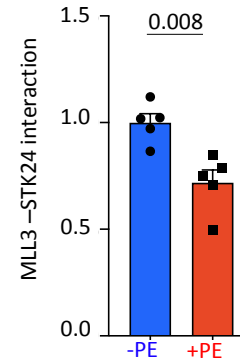
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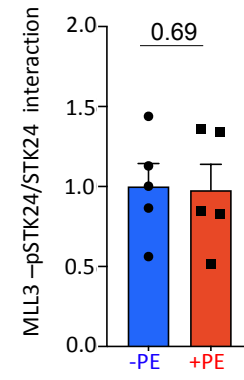
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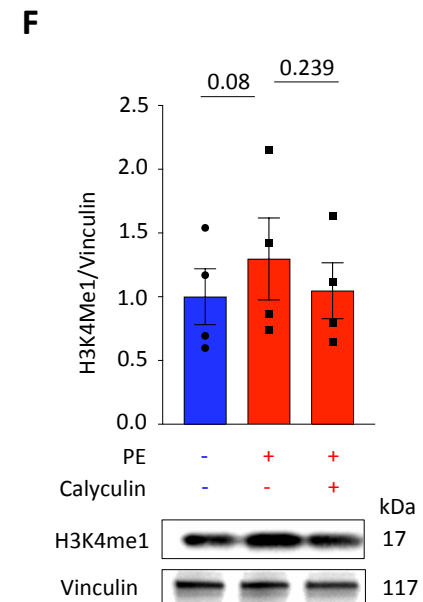
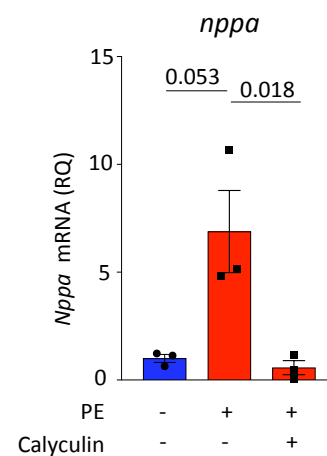
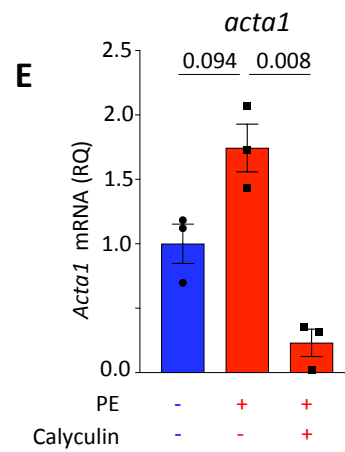
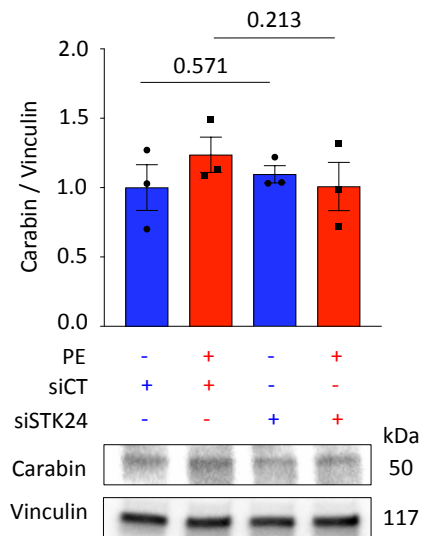
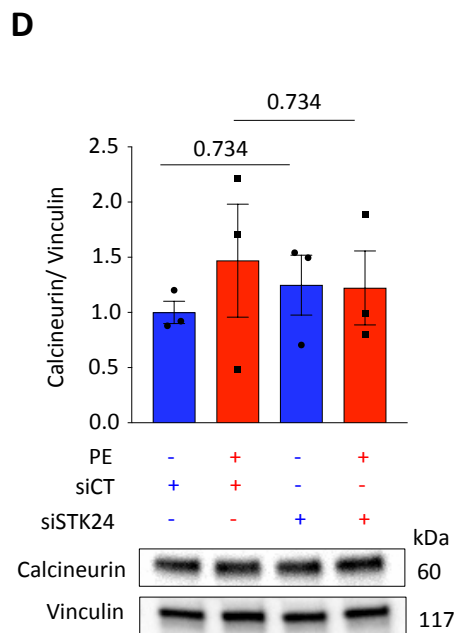
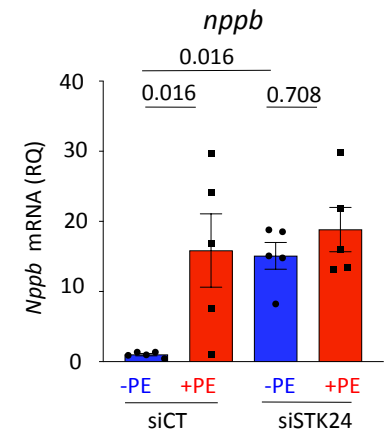
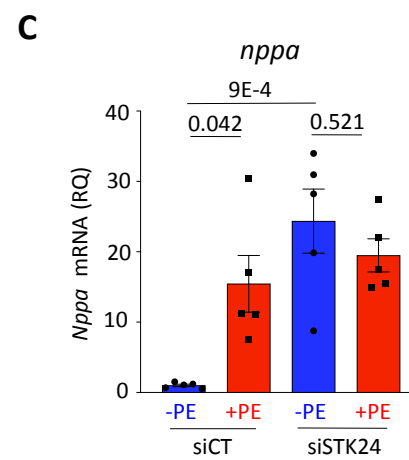
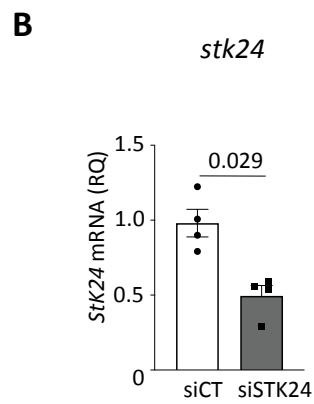
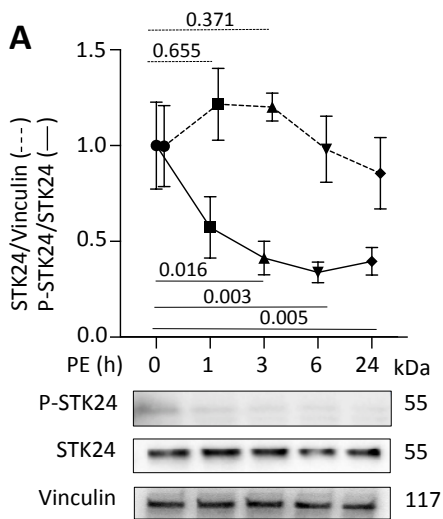
F



Supplemental Figure 4.

A, Immunoprecipitation (IP) experiments followed by immunoblot showing the interaction of Carabin (Carab) or MLL3 with MLL3, Carabin (Carab), calcineurin (CaN), and STK24 in mouse cardiac samples subjected or not (Sham) to one-week TAC (n=2). **B**, MLL3-dependent serine phosphorylation (P-Ser) in ventricular samples subjected or not (Sham) to one-week TAC (n=2) **C**, Representative immunoblot of MLL3 immunoprecipitation experiment in isolated adult rat cardiomyocytes treated or not with PE (10 μ M, 30 min) (n=5). **D-F**, Histograms showing the ratio of immunoprecipitated MLL3 to immunoprecipitated Calcineurin (D), STK24 (E) and phosphorylated ratio of STK24 (F) in isolated adult rat cardiomyocytes submitted or not to PE (10 μ M, 30 min) (n=5). Results are expressed as mean \pm SEM relative to -PE condition. Differences between groups were analyzed by a Mann-Whitney U test (D, E, F). Black lines indicate compared groups with the corresponding p-value. Differences were considered significant when $P < 0.05$.

Supplemental Figure 5

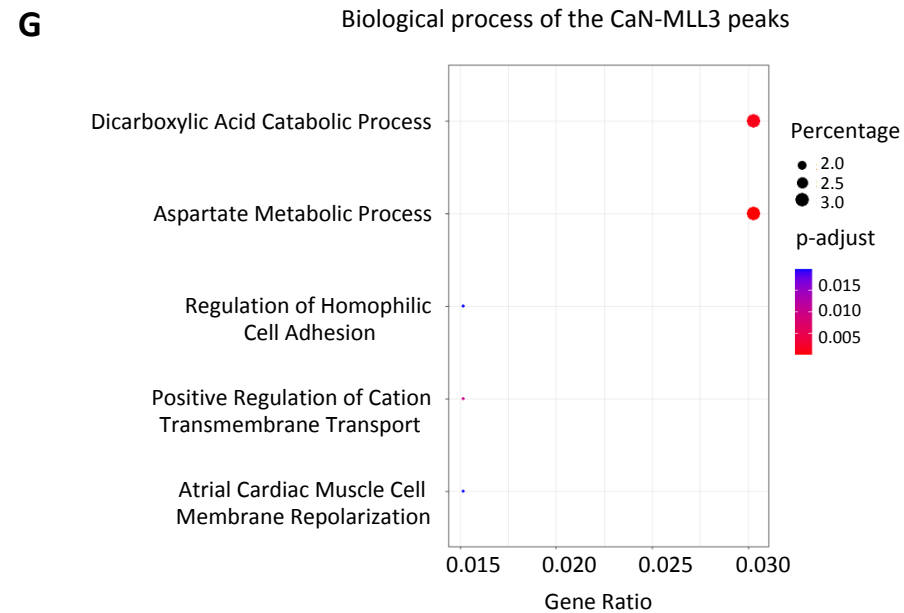
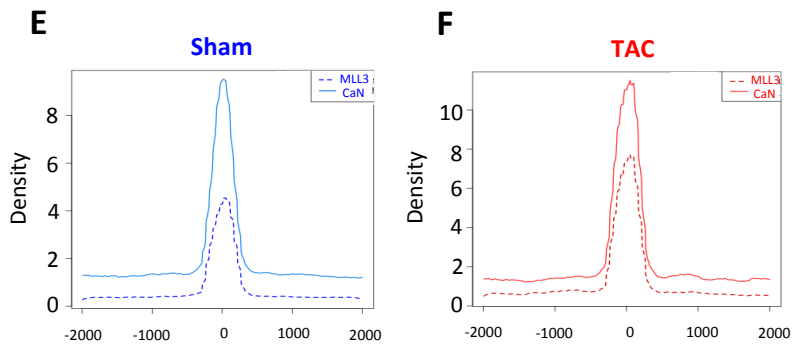
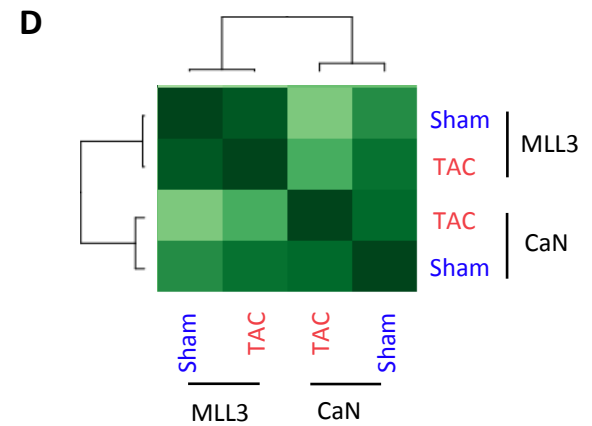
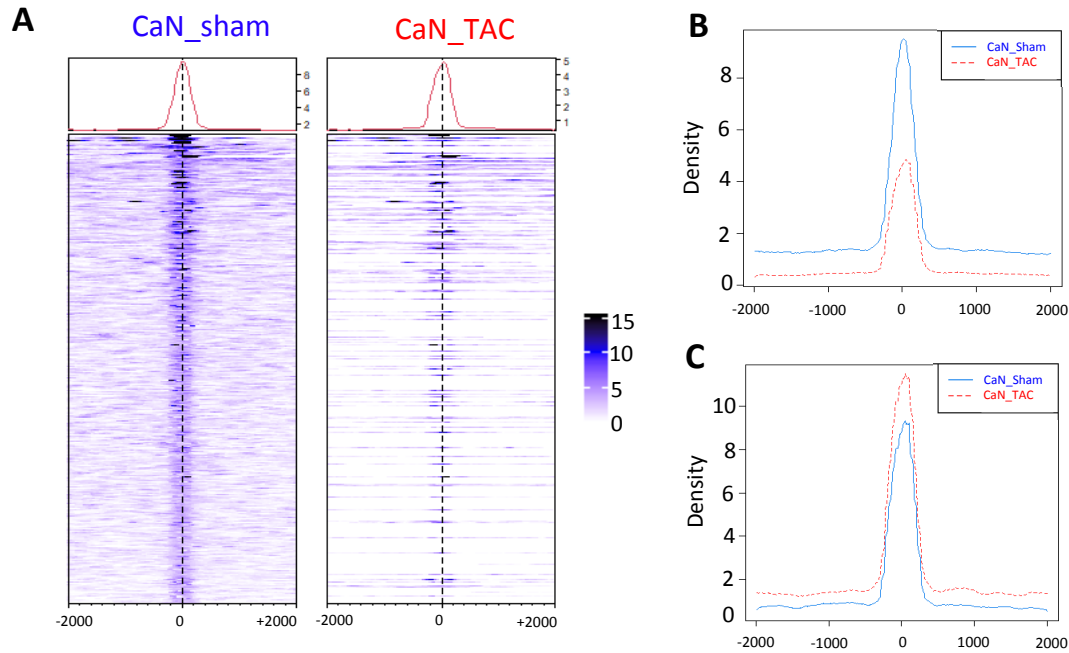


Supplemental Figure 5.

A, Quantification of STK24 and phosphorylated STK24 protein levels in NRVMs treated with PE (10 μ M) for the indicated time (n=5). Representative immunoblots are shown. **B**, Relative expression of *stk24* mRNA in NRVM transfected with either a STK24-targeted siRNA (siSTK24) or a siRNA control (siCT) (n=4). **C**, Relative mRNA expression of the hypertrophic markers *Nppa* and *Nppb* in siCT or siSTK24-transfected NRVMs treated or not with PE (10 μ M, 24 h) (n=5). **D**, Quantification and representative immunoblots of Calcineurin and Carabin protein levels in NRVMs transfected with STK24-targeted (siSTK24) or control (siCT) siRNA and treated or not with PE (10 μ M, 3 h) (n=3). **E**, Relative expression of cardiomyocyte hypertrophy gene markers (*acta1*, *nppa*) in NRVM treated (+) or not (-) with PE (10 μ M, 24 h) and Calyculin (50 nM, 10 min) (n=3). **F**, Quantification and representative immunoblot showing the effect of Calyculin (50 nM, 10 min) on H3K4Me1 level in NRVMs treated or not with PE (10 μ M, 3 h) (n=4).

Results are expressed as mean \pm SEM and normalized to minus PE (A, E, F) and/or siCT (B, C, D). Differences between groups were analyzed by a Friedman test with a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli (A) or a Mann-Whitney U test (B) or a Kruskal-Wallis test with a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli (C, D, E, F). Black lines indicate compared groups with the corresponding p-values. Differences were considered significant when $P < 0.05$.

Supplemental Figure 6



Supplemental Figure 6.

A, Heatmap showing that 31% of calcineurin binding regions in Sham condition show binding signal intensity for Calcineurin in TAC. **B**, Signal intensity plot represented calcineurin binding sites in TAC to calcineurin binding sites in Sham. **C**, Signal intensity plot represented Calcineurin binding sites in Sham to Calcineurin binding sites in TAC. **D**, Hierarchical clustering analysis of calcineurin and MLL3 binding sites in normal (Sham) and hypertrophic (TAC) cardiomyocytes. **E-F**, Signal intensity plot represented colocalisation of MLL3 binding sites to calcineurin binding sites in Sham (E) and TAC (F) conditions. **G**, Gene Ontology analysis showing the biological processes associated with the genes located closest to Sham and TAC CaN and MLL3 cobound peaks.