

Supplemental data for:

Targeting MRTF/SRF in CAP2-dependent dilated cardiomyopathy
delays disease onset

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Supplementary figure legends

Supplementary Figure 1 Gene expression and pathway changes in CAP2 KO hearts as determined by RNA-seq with differentially expressed genes chosen with FDR < 0.2 and fold change of at least 1.2 as indicated by the dashed lines. **(A)** Volcano plots of gene expression in CAP2 KO mice and CAP2 CKO mice. **(B)** SRF regulated genes are activated in CAP2 mutant hearts.

Supplementary Figure 2 ECHO parameters in CAP2 KO mice. **(A)** Ejection fraction and Fractional shortening in CAP2 KO mice at 3 weeks old (n=6) and 13 weeks old (n=6) compared with age-matched controls (n=5~6). Data is presented as mean±SEM. P value compared with WT mice was determined using the 2-tailed paired t test.

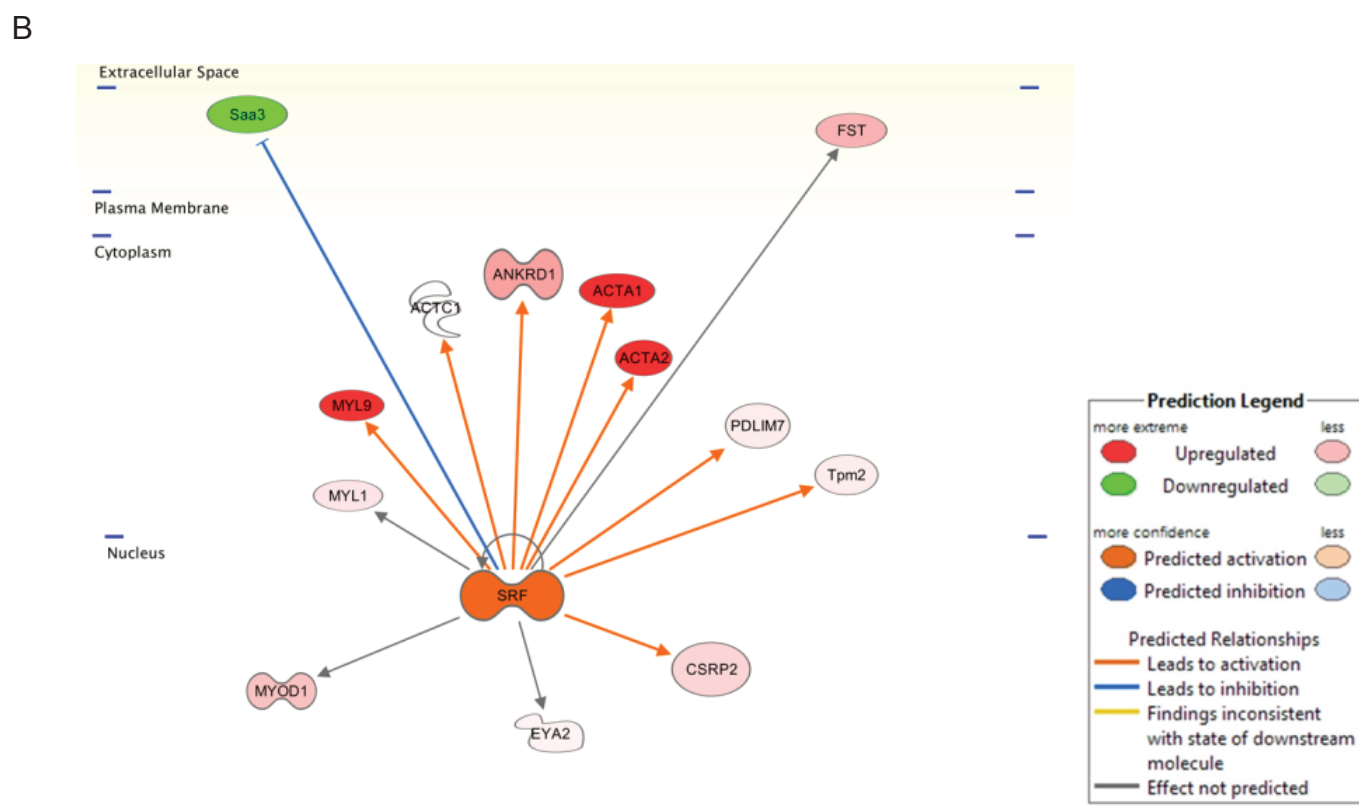
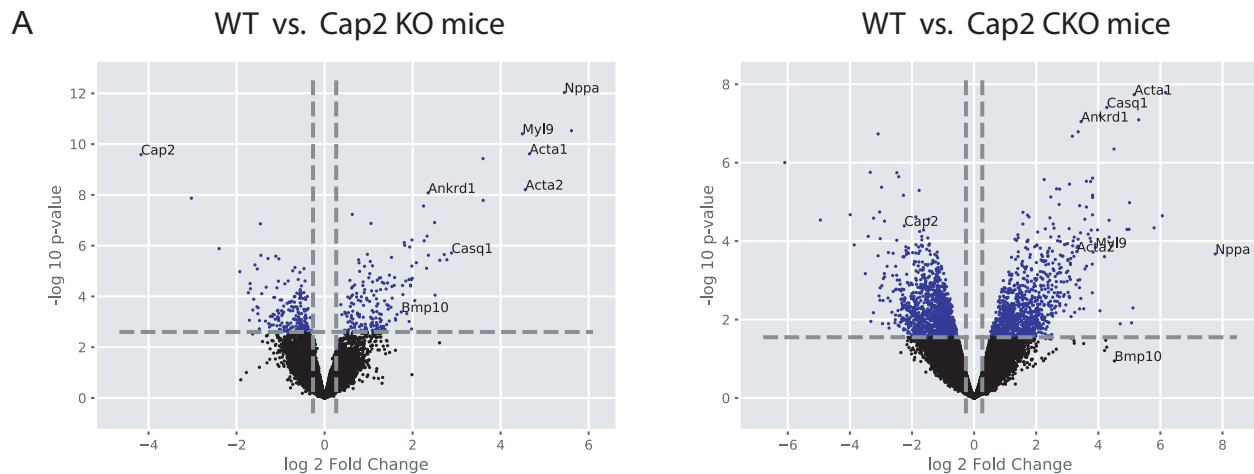
Supplementary Figure 3 SRF gene expression is activated by CAP loss in Hela cells. **(A)** CAP1, CAP2 and ACTA2 expression in CAP1 knockdown Hela cells. **(B)** Knock down of CAP2 in CAP1 knockdown Hela cells using siRNA stimulates ACTA2 expression. Data is presented as mean±SEM. P value compared with WT mice was determined using the 2-tailed paired t test. Three technical triplicates were used in each sample.

Supplementary Figure 4 CAP2 loss does not activate SRF genes in fibroblasts and lack of significant fibrosis in CAP2 CKO hearts. **(A)** Expression of the indicated genes in CAP2 KO MEF cells. Data is presented as mean±SEM. P value compared with WT mice was determined using the 2-tailed paired t test. Three technical triplicates were used in each sample. **(B)** Sirius Red staining of mouse hearts.

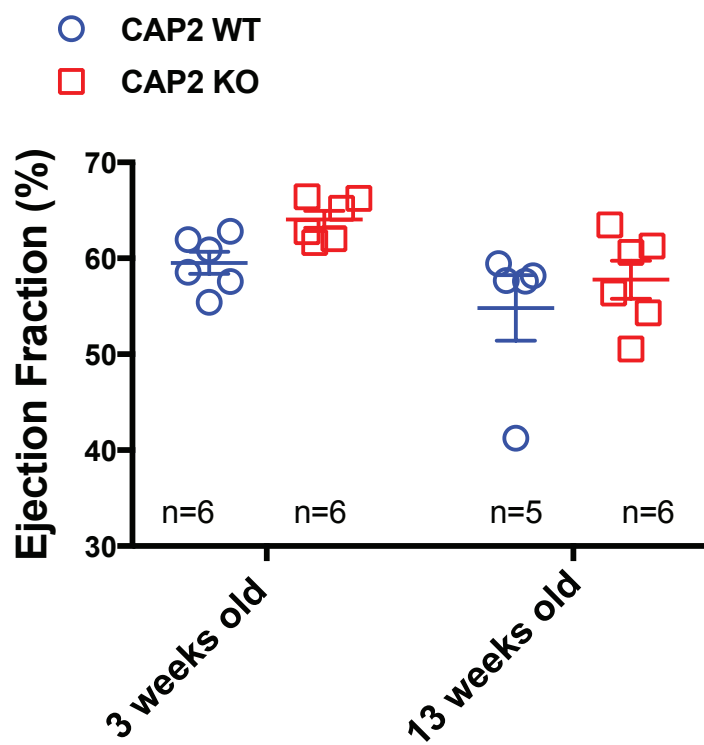
Supplementary Figure 5 Activation of SRF genes in CAP2 CKO mice. **(A)** Scheme for cardiac specific knockouts, CAP2 CKO mice. **(B)** Expression of CAP2 in the hearts from CAP2 CKO mice. **(C)** Expression of SRF genes in the hearts from CAP2 CKO mice. Data is presented as mean±SEM. P value compared with WT mice was determined using the 2-tailed paired t test. Three technical triplicates were used in each sample.

Supplementary Figure 6 CCG-1423-8u inhibits translocation of MRTFA in MEF cells, determined by immune-staining.

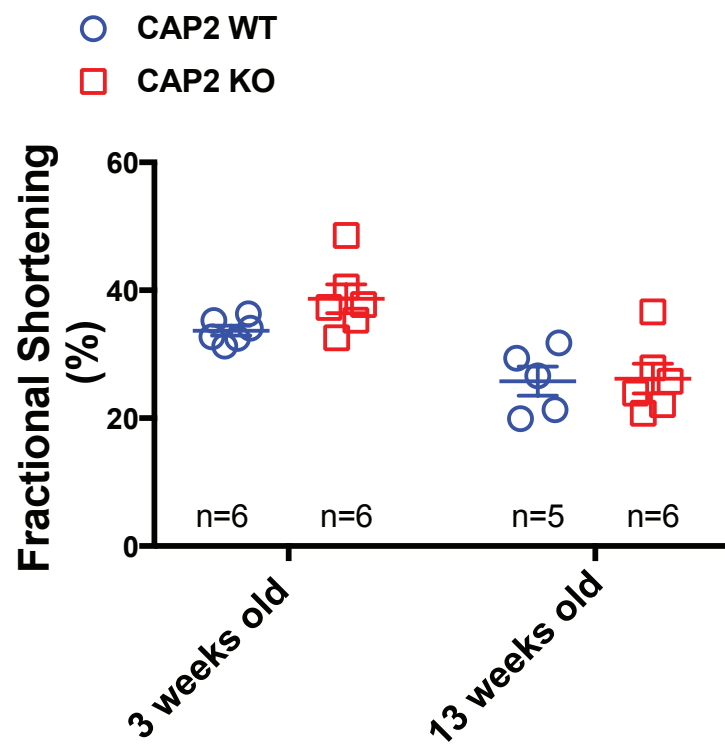
Supplementary Figure 7 Synthesis and characterization of the SRF inhibitor CCG-1423-8u. LRMS data was collected on a Waters Acquity UPLC equipped with SQD with electrospray ionization (ESI) in the positive mode. ¹H NMR data was collected using a Bruker DRX 500 spectrometer at 500 MHz. The sample was dissolved in CDCl₃.



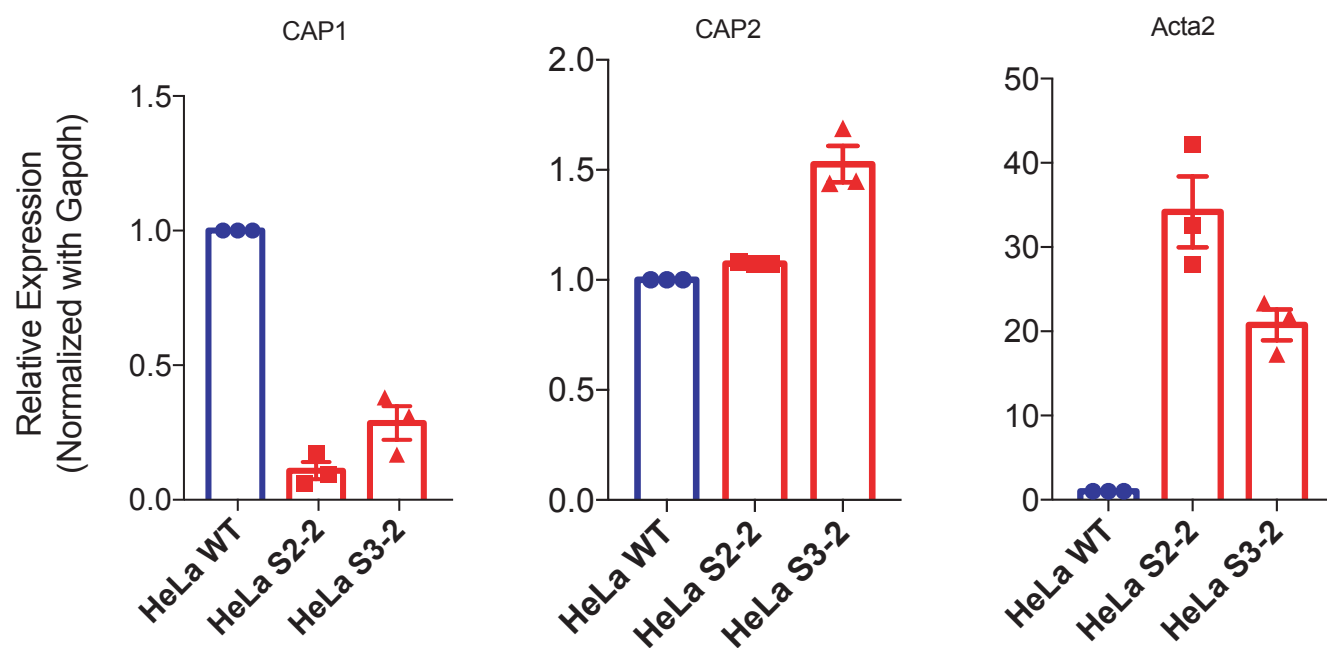
A



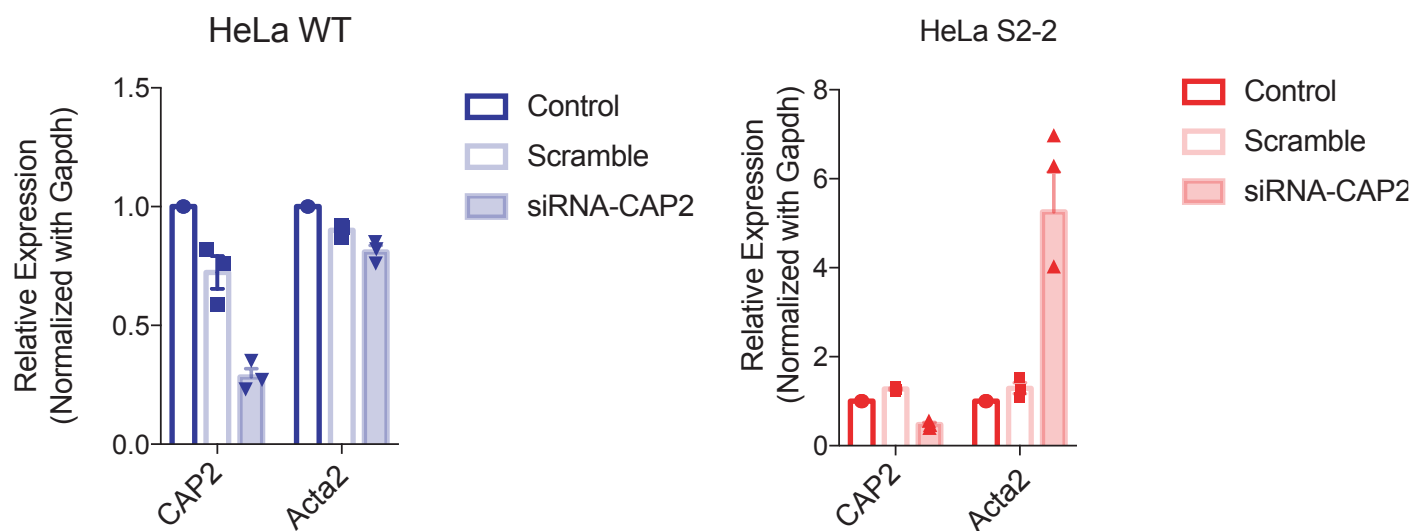
Supplementary Figure 2



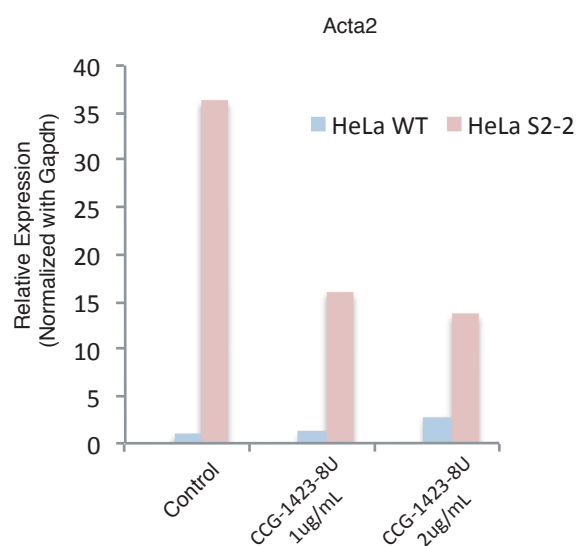
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B

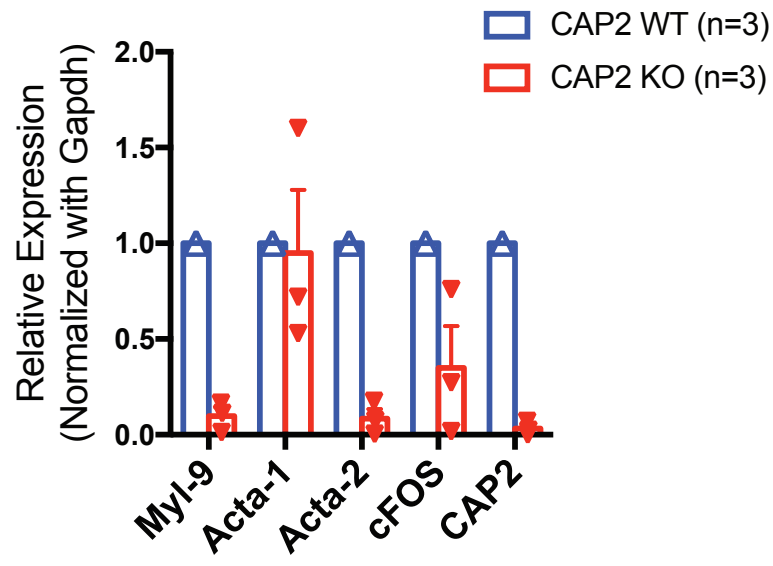


C



Supplementary Figure 4

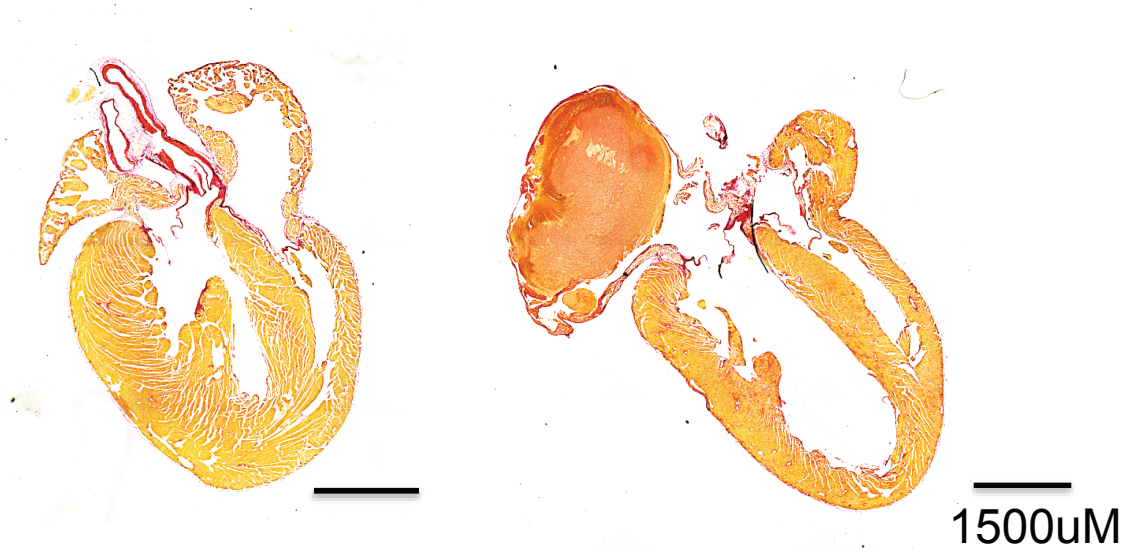
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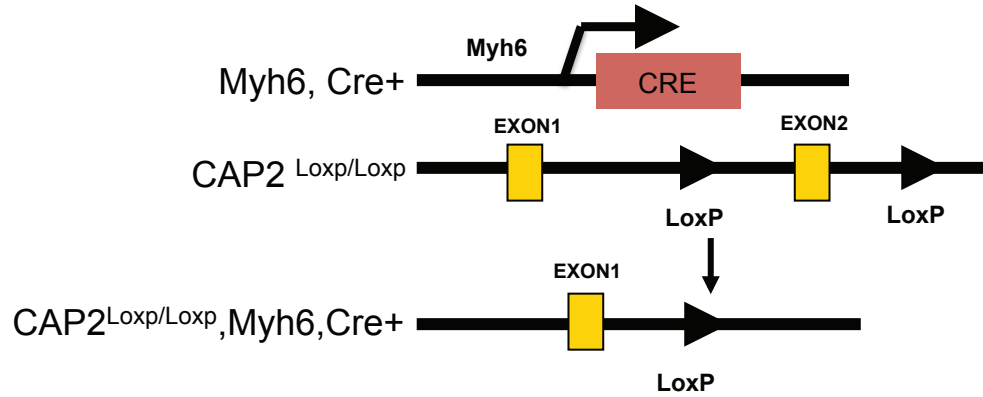
CAP2 WT

CAP2 CKO

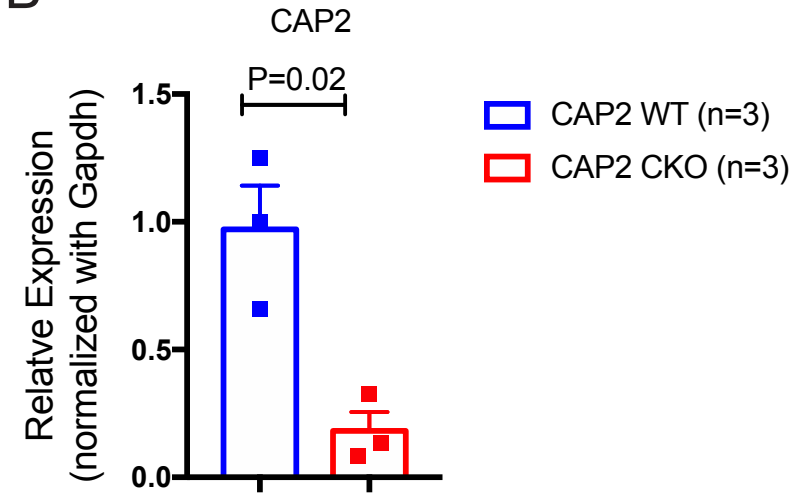


Supplementary Figure 5

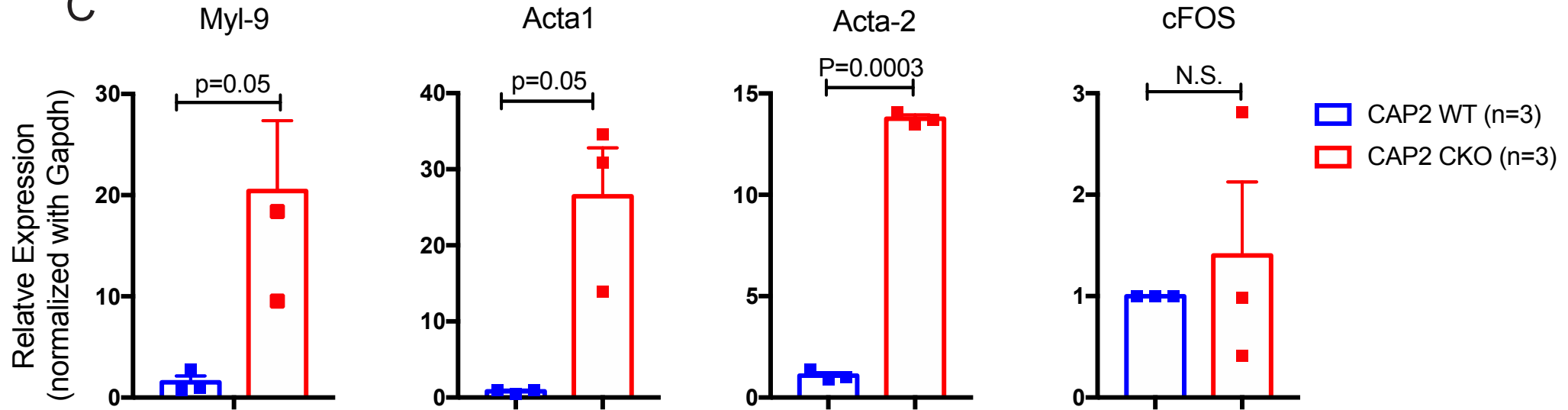
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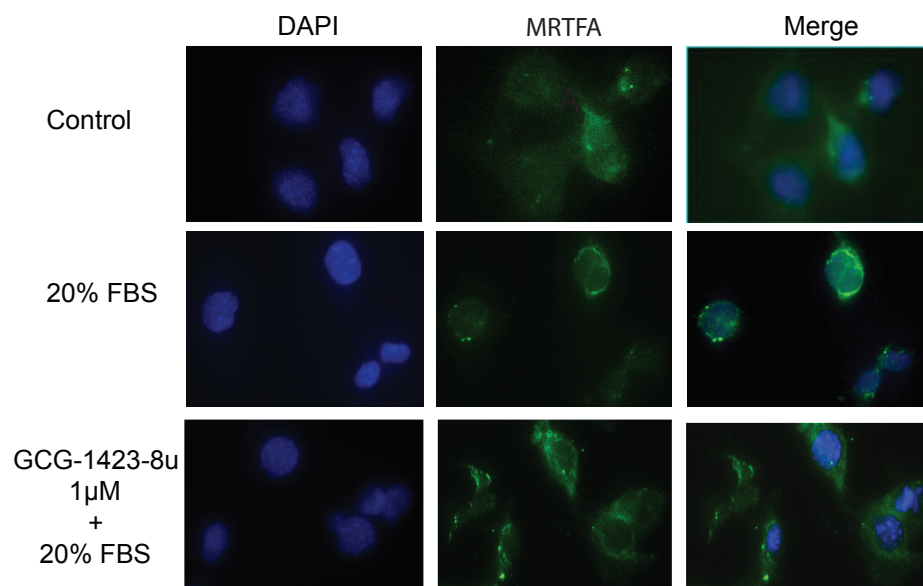


B



C





Supplementary Figure 7

Peak # Target ... BPM(+)
12 cog-142... 425.1

