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Analysis

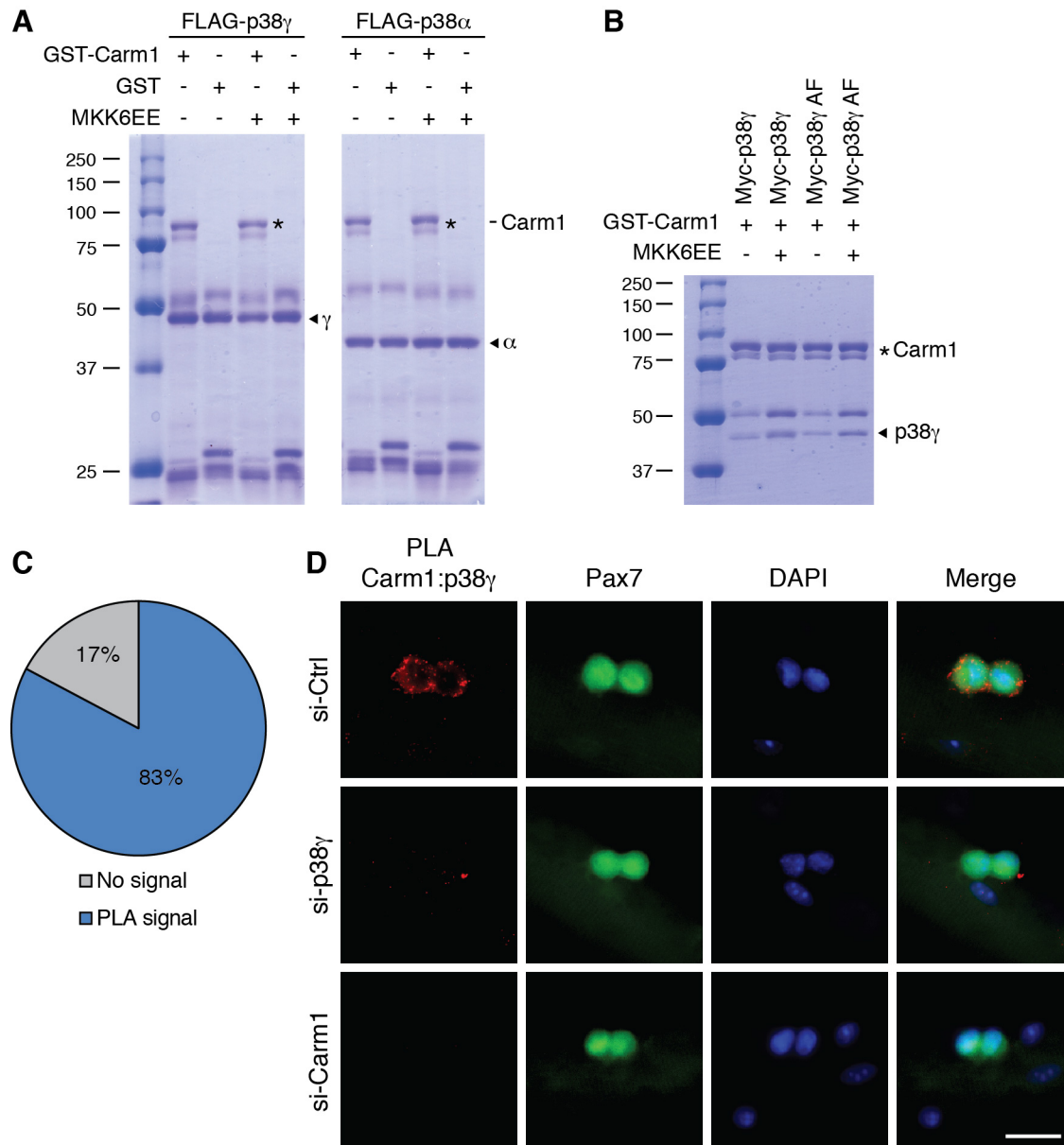


Figure S1. Related to Figure 1. Carm1 is a Substrate for p38 γ

(A) Coomassie blue stained protein gels corresponding to the *in vitro* kinase assay performed in Figure 1A. Asterisks denote GST-Carm1 protein and arrowheads denote FLAG-p38 γ (γ) and FLAG-p38 α (α) kinases.

(B) Coomassie blue stained protein gel corresponding to the *in vitro* kinase assay performed in Figure 1B. The asterisk denotes GST-Carm1 protein and arrowhead denotes Myc-p38 γ kinase protein.

(C) Quantification of the percentage of activated satellite cells (after 42h of culture) that exhibited a Carm1:p38 γ PLA signal.

(D) Carm1:p38 γ PLA (red) performed on satellite cells cultured on isolated single myofibers treated with the indicated siRNAs. Satellite cells are marked by expression of Pax7 (green) and nuclei were counterstained with DAPI (blue). Scale bar represents 20 μ m.

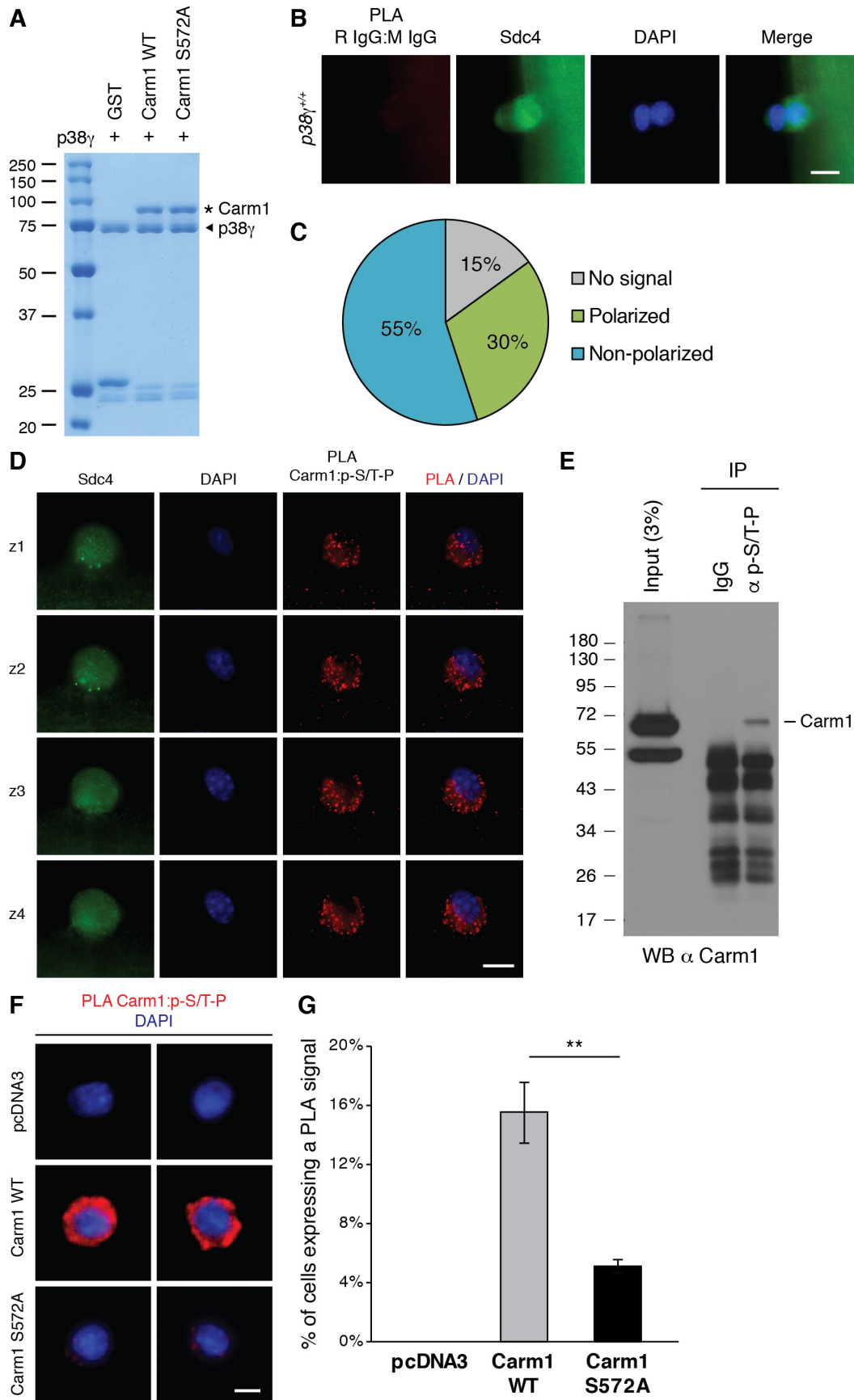


Figure S2. Related to Figure 2. Carm1 is Phosphorylated on S572 by p38 γ

(A) Coomassie blue stained protein gel corresponding to the *in vitro* kinase assay performed in Figure 2E. The asterisk denotes Carm1 protein and arrowhead denotes active p38 γ kinase.

(B) Control PLA with rabbit and mouse IgG (red) performed on satellite cells cultured on single myofibers isolated from *p38 γ ^{+/+}* mice. Satellite cells are marked by expression of Syndecan 4 (Sdc4, green) and nuclei were counterstained with DAPI (blue). Scale bar represents 10 μ m.

(C) Quantification of the percentage of activated satellite cells (cultured for 36h) exhibiting either no signal, or a polarized or non-polarized Carm1:p-S/T-P PLA signal.

(D) Z-stack images of a satellite cell expressing a Carm1:p-S/T-P PLA signal (red) and Sdc4 (green). The nucleus was counterstained with DAPI (blue). Images were acquired with a step size of 0.8 μ m. Scale bar represents 10 μ m.

(E) Immunoprecipitations (IP) with anti-p-S/T-P antibody or mouse IgG control were performed on cell lysates made from satellite cell-derived primary myoblasts. Immunoblot analysis was performed with anti-Carm1 antibody.

(F) *Carm1^{-/-}* primary myoblasts were transiently transfected with either wild type Carm1, Carm1 S572A mutant, or pcDNA3 vector control. PLA (red) was performed with anti-Carm1 and anti-p-S/T-P antibodies and nuclei were counterstained with DAPI (blue). Scale bar represents 5 μ m.

(G) Quantification of the percentage of cells expressing a Carm1:p-S/T-P PLA signal in (F), represented as the mean (n = 5) \pm SEM (**p \leq 0.01).

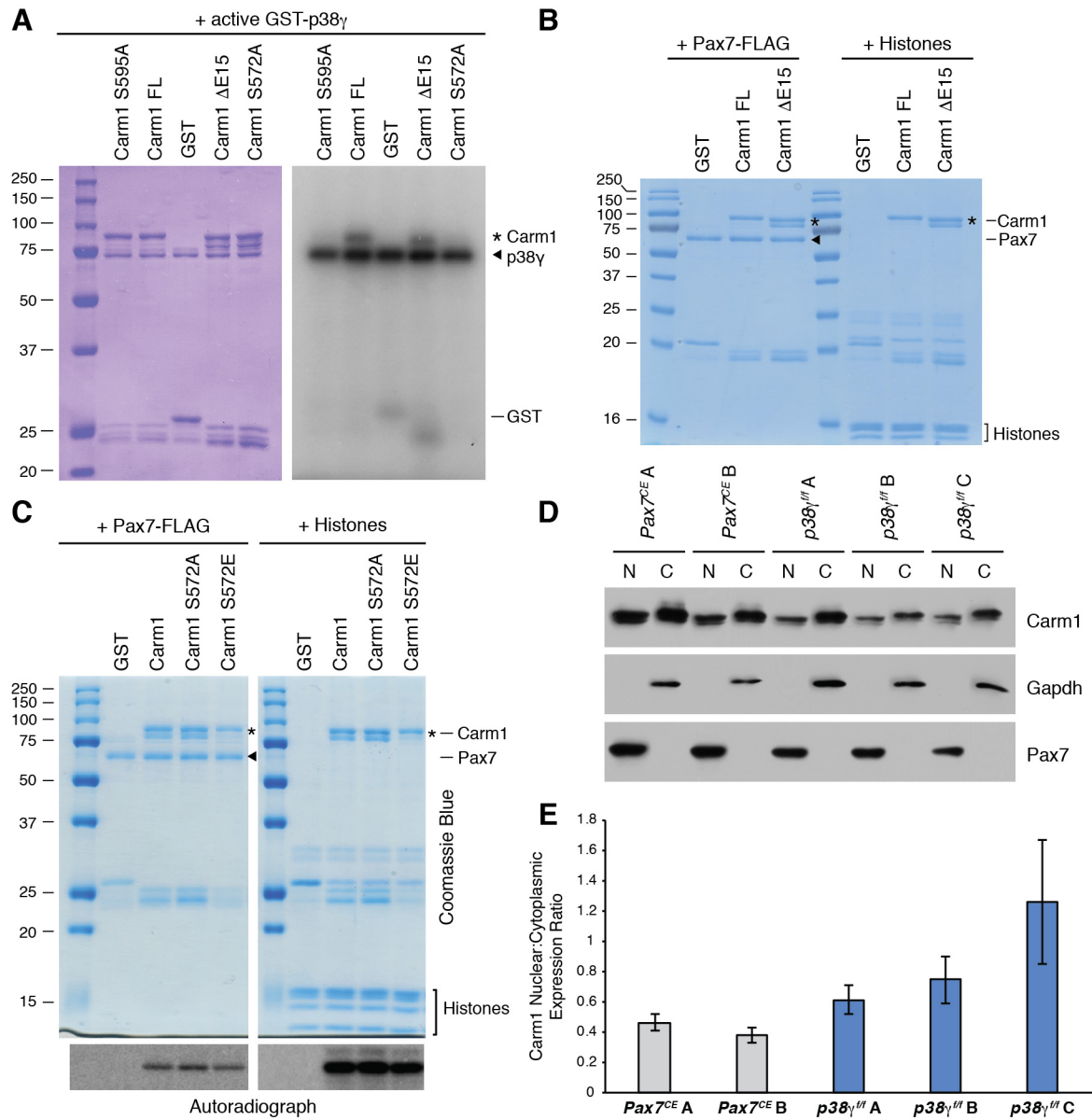


Figure S3. Related to Figure 3. Carm1 Isoform 2 is Functionally Regulated by p38 γ

(A) *In vitro* kinase assays between Carm1 FL (wild type and S595A mutant) or Carm1 Δ E15 (wild type and S572A mutant) with active p38 γ kinase. The asterisk denotes Carm1 protein and arrowhead denotes active p38 γ kinase.

(B) Coomassie blue stained protein gel corresponding to the *in vitro* methylation assay performed in Figure 3B. The asterisks denote Carm1 protein, and the arrowhead denotes Pax7 protein.

(C) *In vitro* methylation assays with wild type Carm1, Carm1 S572A and S572E mutants with either Pax7 or core histone proteins. The asterisks denote Carm1 protein and the arrowhead denotes Pax7 protein.

(D) Subcellular fractionation of satellite cell-derived primary myoblasts from $Pax7^{CreER/+}$ ($Pax7^{CE}$) and $Pax7^{CreER/+};p38^{\Delta/fl}$ ($p38^{\Delta/fl}$) mice. Immunoblot analysis was performed with the indicated antibodies.

(E) Densitometric quantification of Carm1 expression levels from (D), expressed as a ratio of nuclear to cytoplasmic expression. Carm1 expression was normalized to nucleus and cytoplasm loading controls (Pax7 and Gadph, respectively).

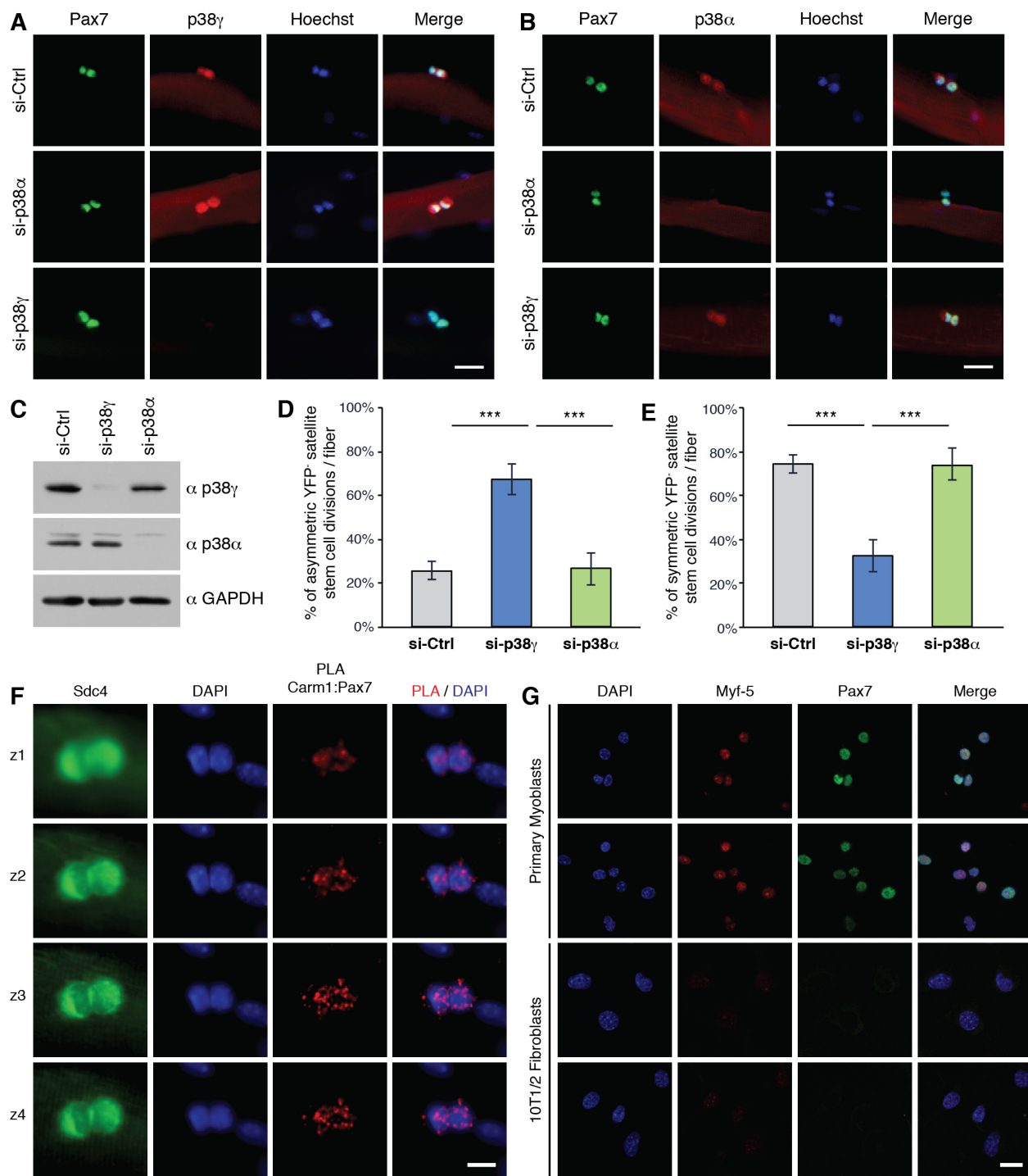


Figure S4. Related to Figure 4. Validation of p38 γ and p38 α siRNAs and Supplementary Analyses

(A and B) Immunofluorescence staining with the indicated antibodies of satellite cells cultured for 42h on myofibers that were treated with the indicated siRNAs. Scale bar represents 20 μ m.

(C) Immunoblot analysis with the indicated antibodies of satellite cell-derived primary myoblasts treated with the indicated siRNAs.

(D) Quantification of the percentage of satellite stem cell (Pax7⁺/YFP⁻) divisions that are asymmetric, represented as the mean (n = 9) ± SEM (**p ≤ 0.001).

(E) Quantification of the percentage of satellite stem cell (Pax7⁺/YFP⁻) divisions that are symmetric, represented as the mean (n = 9) ± SEM (**p ≤ 0.001).

(F) Z-stack images of satellite cells expressing a Carm1:Pax7 PLA signal (red) and Sdc4 (green). Nuclei were counterstained with DAPI (blue). Images were acquired with a step size of 0.8 μm. Scale bar represents 10 μm.

(G) Validation of the anti-Myf5 antibody used in Figure 4D. Representative immunofluorescence images of primary myoblasts and 10T1/2 fibroblasts immunostained with Pax7 (green) and Myf5 (red). Nuclei were counterstained with DAPI (blue). Scale bar represents 20 μm.

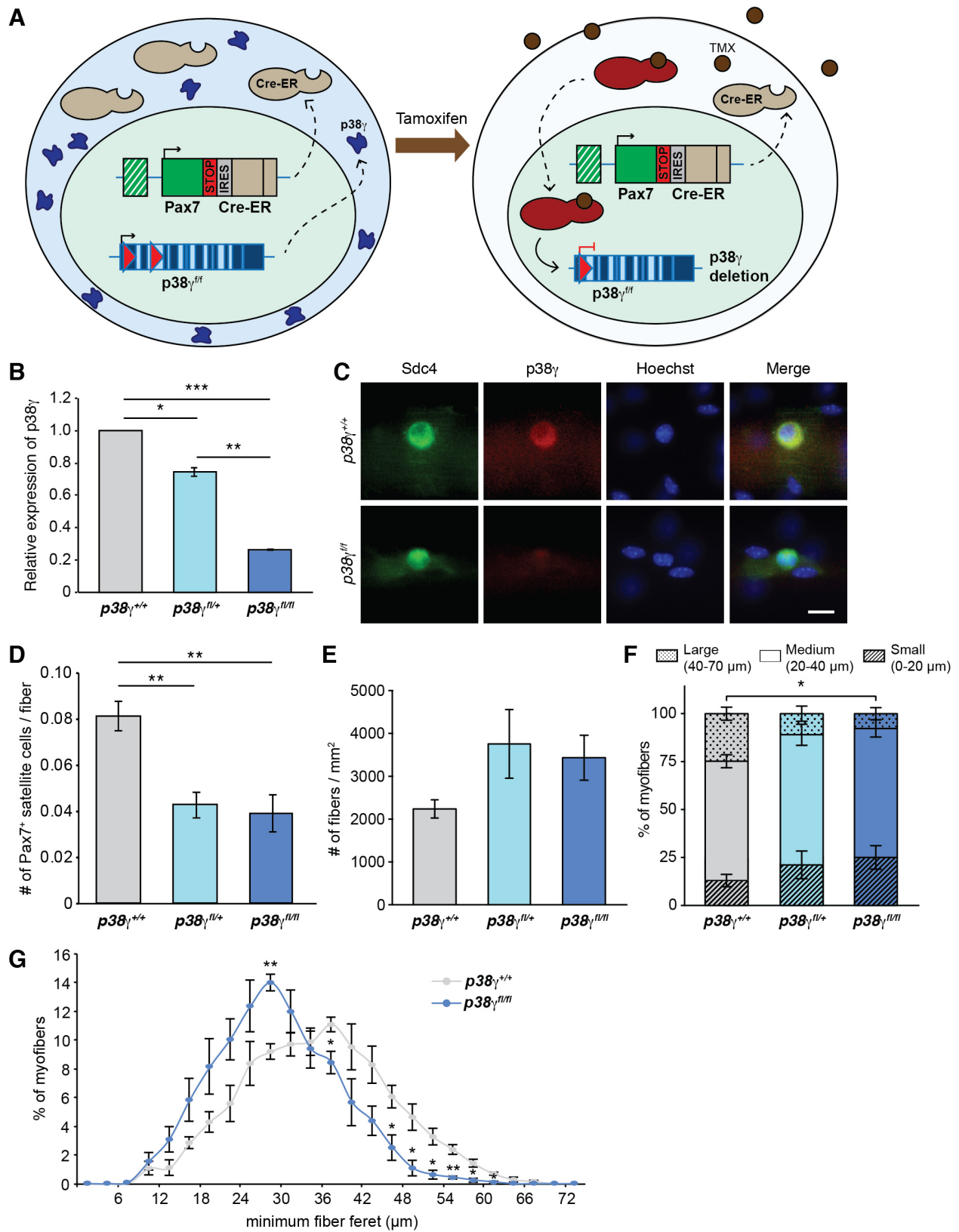


Figure S5. Related to Figure 5. Description and Validation of the *Pax7^{CreER/+}·p38^{γ^{fl/fl}} Mouse Model*

(A) Schematic illustrating the satellite cell-specific genetic deletion of p38 γ upon administration of tamoxifen (TMX). Following TMX administration, activated CreER-recombinase translocates to the nucleus to induce excision at the loxP sites (red arrowheads) within the *p38 γ* gene resulting in genetic deletion of *p38 γ* in *Pax7*-expressing cells.

(B) Relative expression of *p38 γ* in prospectively isolated satellite cells from TMX-treated *p38 $\gamma^{+/+}$* , *p38 $\gamma^{fl/+}$* and *p38 $\gamma^{fl/fl}$* mice as determined by ddPCR. Data presented as the mean (n = 3) \pm SEM (*p \leq 0.05, **p \leq 0.01, ***p \leq 0.001).

(C) Immunostaining with the indicated antibodies of myofibers isolated from TMX-treated *p38 $\gamma^{+/+}$* and *p38 $\gamma^{fl/fl}$* mice and cultured for 36h. Scale bar represents 10 μ m.

(D) Quantification of Pax7⁺ satellite cells normalized to total fiber number from CTX-injured TA muscles isolated from mice described in Figure 5A, represented as the mean (n = 4 *p38 $\gamma^{+/+}$* , n = 3 *p38 $\gamma^{fl/+}$* , n = 3 *p38 $\gamma^{fl/fl}$*) \pm SEM (**p \leq 0.01).

(E) Quantification of the number of fibers normalized to TA cross-sectional area, represented as the mean (n = 4 *p38 $\gamma^{+/+}$* , n = 3 *p38 $\gamma^{fl/+}$* , n = 3 *p38 $\gamma^{fl/fl}$*) \pm SEM.

(F) Minimal fiber Feret was determined from 500 centrally-nucleated fibers per TA cross section, represented as the mean percentage of fibers that are small (0-20 μ m), medium (20-40 μ m) or large (40-70 μ m) \pm SEM (*p \leq 0.05).

(G) Fiber size distribution, as determined in (F) (*p \leq 0.05, **p \leq 0.01).

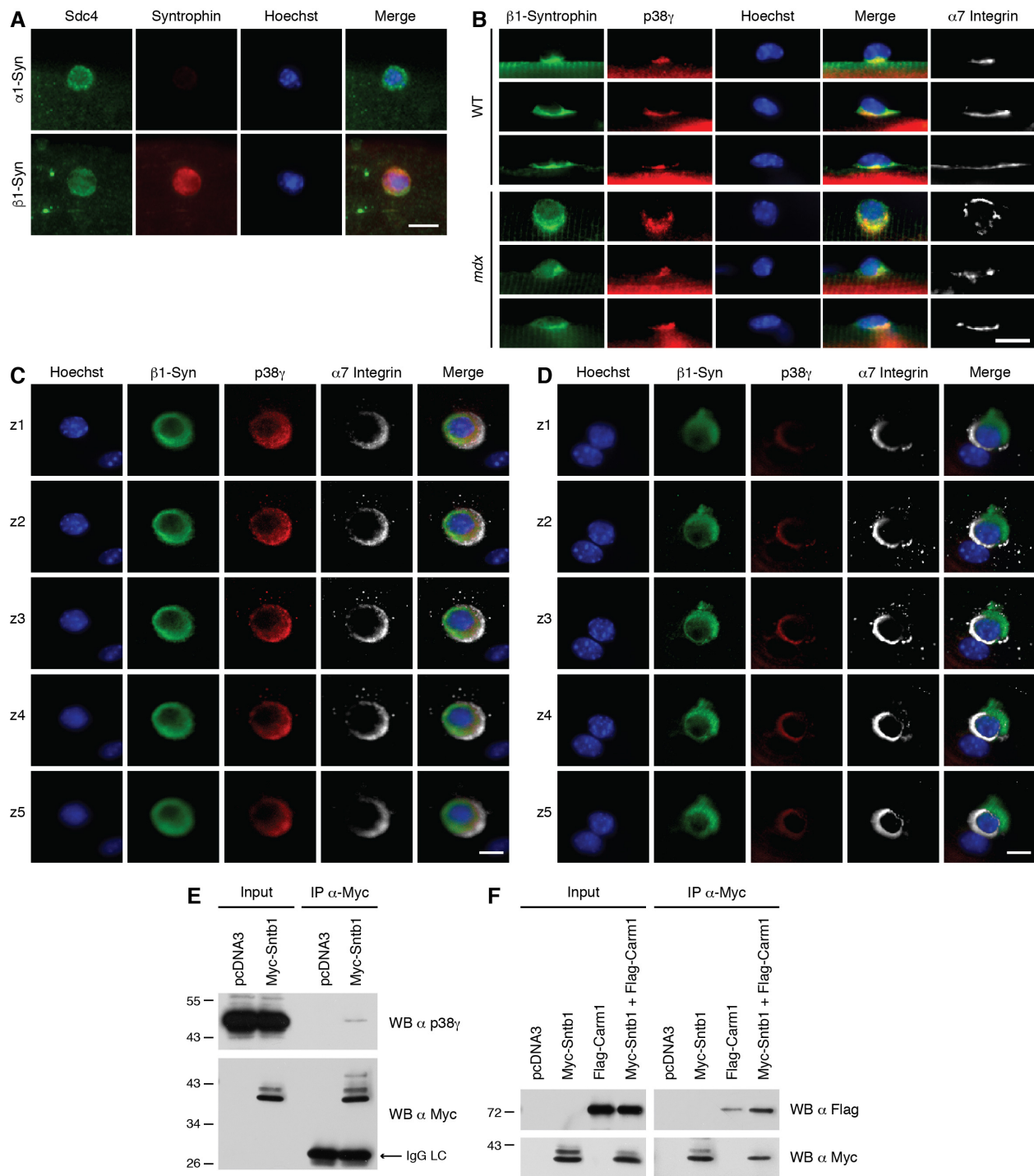


Figure S6. Related to Figure 6. p38 γ /Carm1 Interacts with $\beta 1$ -Syntrophin in Satellite Cells

(A) Immunofluorescence analysis of syntrophin isoform expression in satellite cells. Myofibers cultured for 36h were stained with antibodies against either $\alpha 1$ -syntrophin or $\beta 1$ -syntrophin

(red). Satellite cells are marked by Sdc4 expression (green) and nuclei were counterstained with Hoechst (blue). Scale bar represents 10 μm .

(B) Immunofluorescence of satellite cells cultured for 36h on myofibers isolated from wild type (WT) or *mdx* mice. Cells were immunostained for $\alpha 7$ integrin (white), $\beta 1$ -syntrophin (green), and p38 γ (red). Nuclei were counterstained with Hoechst (blue). Scale bar represents 10 μm .

(C) Z-stack images of a wild type satellite cell expressing $\beta 1$ -syntrophin (green), p38 γ (red), and $\alpha 7$ Integrin (white). Nuclei were counterstained with Hoechst (blue). Images were acquired with a step size of 0.4 μm . Scale bar represents 10 μm .

(D) Z-stack images of a dystrophin-deficient *mdx* satellite cell expressing $\beta 1$ -syntrophin (green), p38 γ (red), and $\alpha 7$ Integrin (white). Nuclei were counterstained with Hoechst (blue). Images were acquired with a step size of 0.4 μm . Scale bar represents 10 μm .

(E) HEK 293T cells were transfected with Myc-tagged $\beta 1$ -syntrophin (Myc-Sntb1).

Immunoprecipitation (IP) and immunoblotting were performed with the indicated antibodies.

(F) HEK 293T cells were transfected with Myc-tagged $\beta 1$ -syntrophin (Myc-Sntb1) and Flag-tagged Carm1 (Flag-Carm1). Immunoprecipitation (IP) and immunoblotting were performed with the indicated antibodies.

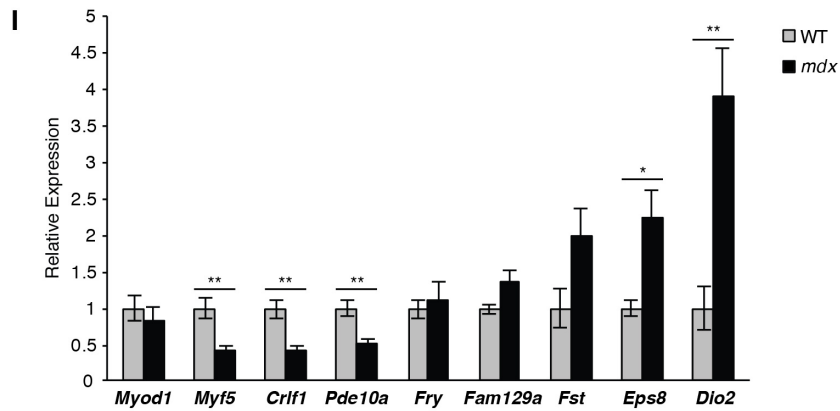
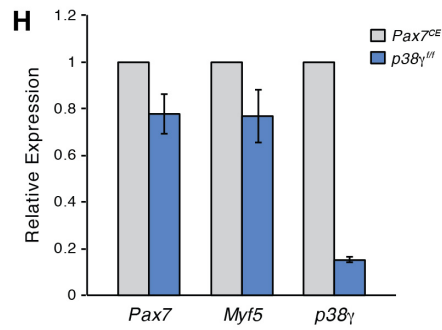
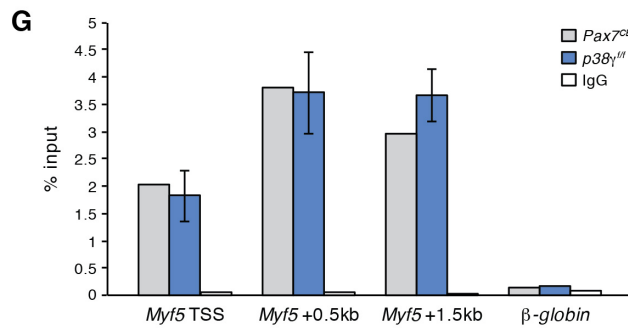
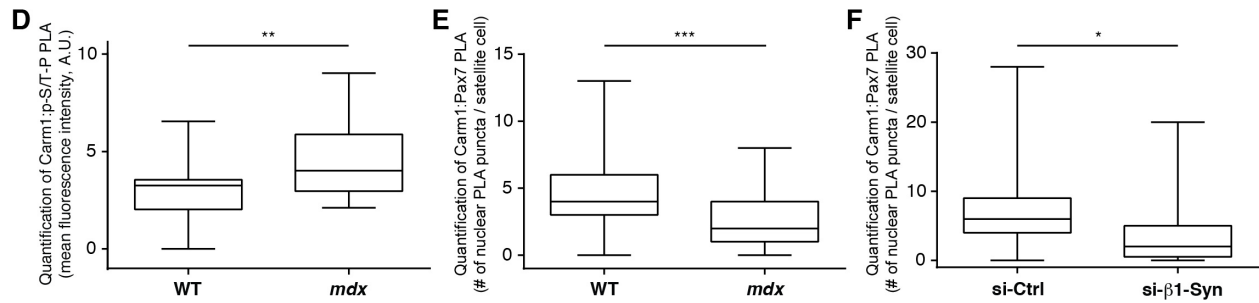
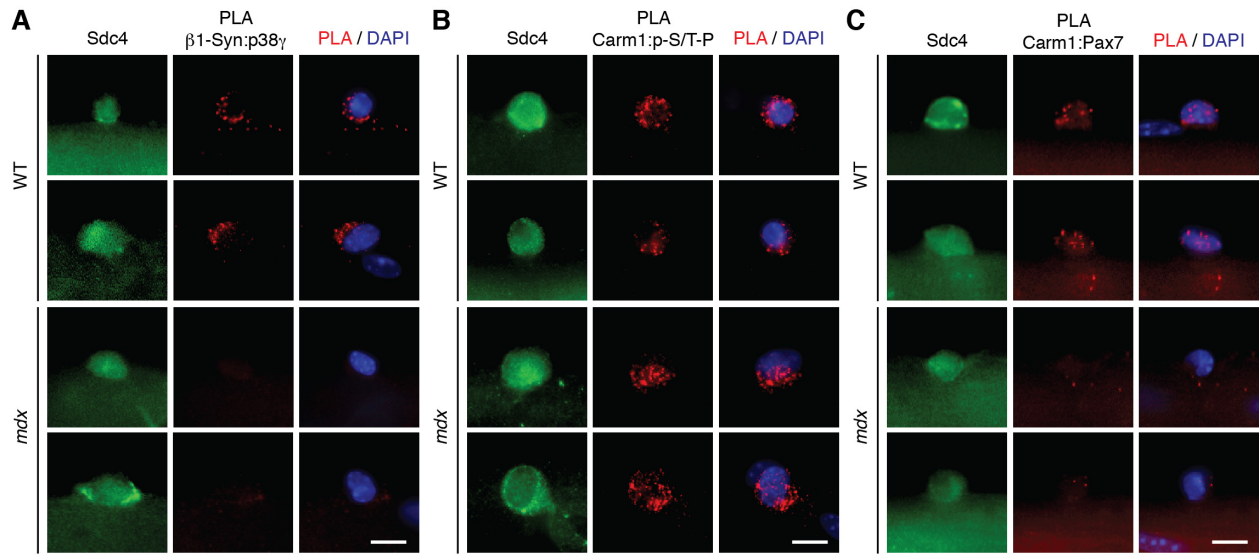


Figure S7. Related to Figures 6 and 7. p38 γ -Mediated Carm1 Regulation is Perturbed in *mdx* Satellite Cells

(A, B, and C) Additional examples of PLA (red) with indicated antibodies performed on 36h cultured myofibers isolated from wild type (WT) and *mdx* mice. Satellite cells are marked by Sdc4 expression (green) and nuclei were counterstained with DAPI (blue). Scale bar represents 10 μ m.

(D) Box and whiskers plot representation of the quantification of Carm1:p-S/T-P PLA in Figure 6G, represented as the relative mean (n = 21 WT, n = 26 *mdx*) \pm SEM (**p \leq 0.01). The PLA was quantified by determining mean fluorescence intensity of the PLA signal of each satellite cell (A.U., arbitrary units).

(E) Box and whiskers plot representation of the quantification Carm1:Pax7 PLA in Figure 7B, represented as the relative mean (n = 81 WT, n = 137 *mdx*) \pm SEM (**p \leq 0.001). The PLA was quantified by counting the number of nuclear PLA puncta of each satellite cell.

(F) Box and whiskers plot representation of the quantification of Carm1:Pax7 PLA in Figure 7D, represented as the relative mean (n = 53 si-Ctrl, n = 73 si- β 1-Syn) \pm SEM (*p \leq 0.05). The PLA was quantified by counting the number of nuclear PLA puncta of each satellite cell.

(G) qPCR analyses of indicated genes in freshly sorted satellite cells from *Pax7^{CreER/+}* (*Pax7^{CE}*) and *Pax7^{CreER/+}:p38^{fl/fl}* (*p38^{fl/fl}*) mice 3 days post injury with cardiotoxin, represented as the mean (n = 1 *Pax7^{CE}*, n = 4 *p38^{fl/fl}*) \pm SEM.

(H) ChIP-qPCR analysis of H3K4me3 at *Myf5* in *Pax7^{CE}* and *p38^{fl/fl}* primary myoblasts represented as the mean (n = 2 *Pax7^{CE}*, n = 3 *p38^{fl/fl}*) \pm SEM.

(I) qPCR gene expression analysis of indicated Pax7 target genes in freshly sorted satellite cells from WT and *mdx* mice represented as the mean (n = 6 WT, n = 7 *mdx*) \pm SEM (*p \leq 0.05, **p \leq 0.01).

SUPPLEMENTAL TABLES

#	b	b ⁺⁺	b [*]	b ^{***}	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y [*]	y ^{***}	y ⁰	y ⁰⁺⁺	#
1	72.0444	36.5258					A							30
2	171.1128	86.06					V	3185.3084	1593.1578	3168.2818	1584.6446	3167.2978	1584.1525	29
3	285.1557	143.0815	268.1292	134.5682			N	3086.24	1543.6236	3069.2134	1535.1104	3068.2294	1534.6183	28
4	399.1987	200.103	382.1721	191.5897			N	2972.197	1486.6022	2955.1705	1478.0889	2954.1865	1477.5969	27
5	527.2572	264.1323	510.2307	255.619			Q	2858.1541	1429.5807	2841.1276	1421.0674	2840.1436	1420.5754	26
6	674.3257	337.6665	657.2991	329.1532			F	2730.0955	1365.5514	2713.069	1357.0381	2712.085	1356.5461	25
7	775.3733	388.1903	758.3468	379.677	757.3628	379.185	T	2583.0271	1292.0172	2566.0006	1283.5039	2565.0166	1283.0119	24
8	922.4087	461.708	905.3822	453.1947	904.3982	452.7027	M	2481.9794	1241.4934	2464.9529	1232.9801	2463.9689	1232.4881	23
9	979.4302	490.2187	962.4036	481.7055	961.4196	481.2135	G	2334.944	1167.9757	2317.9175	1159.4624	2316.9335	1158.9704	22
10	1036.4517	518.7295	1019.4251	510.2162	1018.4411	509.7242	G	2277.9226	1139.4649	2260.896	1130.9517	2259.912	1130.4596	21
11	1133.5044	567.2558	1116.4779	558.7426	1115.4939	558.2506	P	2220.9011	1110.9542	2203.8746	1102.4409	2202.8906	1101.9489	20
12	1204.5415	602.7744	1187.515	594.2611	1186.531	593.7691	A	2123.8484	1062.4278	2106.8218	1053.9145	2105.8378	1053.4225	19
13	1317.6256	659.3164	1300.5991	650.8032	1299.615	650.3112	I	2052.8112	1026.9093	2035.7847	1018.396	2034.8007	1017.904	18
14	1404.6576	702.8325	1387.6311	694.3192	1386.6471	693.8272	S	1939.7272	970.3672	1922.7006	961.854	1921.7166	961.3619	17
15	1551.693	776.3502	1534.6665	767.8369	1533.6825	767.3449	M	1852.6952	926.8512	1835.6686	918.3379	1834.6846	917.8459	16
16	1622.7301	811.8687	1605.7036	803.3554	1604.7196	802.8634	A	1705.6598	853.3335	1688.6332	844.8202	1687.6492	844.3282	15
17	1789.7285	895.3679	1772.702	886.8546	1771.7179	886.3626	S	1634.6226	817.815	1617.5961	809.3017	1616.6121	808.8097	14
18	1886.7813	943.8943	1869.7547	935.381	1868.7707	934.889	P	1467.6243	734.3158	1450.5977	725.8025	1449.6137	725.3105	13
19	2033.8167	1017.412	2016.7901	1008.8987	2015.8061	1008.4067	M	1370.5715	685.7894	1353.545	677.2761	1352.561	676.7841	12
20	2120.8487	1060.928	2103.8221	1052.4147	2102.8381	1051.9227	S	1223.5361	612.2717	1206.5096	603.7584	1205.5256	603.2664	11
21	2233.9328	1117.47	2216.9062	1108.9567	2215.9222	1108.4647	I	1136.5041	568.7557	1119.4775	560.2424	1118.4935	559.7504	10
22	2330.9855	1165.9964	2313.959	1157.4831	2312.975	1156.9911	P	1023.42	512.2136	1006.3935	503.7004	1005.4095	503.2084	9
23	2432.0332	1216.5202	2415.0066	1208.007	2414.0226	1207.515	T	926.3673	463.6873	909.3407	455.174	908.3567	454.682	8
24	2546.0761	1273.5417	2529.0496	1265.0284	2528.0656	1264.5364	N	825.3196	413.1634	808.293	404.6502	807.309	404.1581	7
25	2647.1238	1324.0655	2630.0973	1315.5523	2629.1132	1315.0603	T	711.2767	356.142			693.2661	347.1367	6
26	2794.1592	1397.5832	2777.1327	1389.07	2776.1486	1388.578	M	610.229	305.6181			592.2184	296.6128	5
27	2931.2181	1466.1127	2914.1916	1457.5994	2913.2076	1457.1074	H	463.1936	232.1004			445.183	223.0951	4
28	3094.2814	1547.6444	3077.2549	1539.1311	3076.2709	1538.6391	Y	326.1347	163.571			308.1241	154.5657	3
29	3151.3029	1576.1551	3134.2764	1567.6418	3133.2923	1567.1498	G	163.0713	82.0393			145.0608	73.034	2
30							S	106.0499	53.5286			88.0393	44.5233	1

Table S1. Related to Figure 2. Fragment Mass Match Table

All predicted MS2 peaks for the indicated peptide sequence as described in Figure 2B (including the indicated oxidations and phosphorylation). Observed peaks are highlighted in red.

Score	Mr(calc)	Delta	Sequence	Site Analysis
64.5	3255.3382	0.0043	AVNNQFTMGGPAISMASPMSIPTNTMHYGS	Phospho S17 95.87%
48.8	3255.3382	0.0043	AVNNQFTMGGPAISMASPMSIPTNTMHYGS	Phospho S20 2.61%
46.3	3255.3382	0.0043	AVNNQFTMGGPAISMASPMSIPTNTMHYGS	Phospho S14 1.48%
29.2	3255.3382	0.0043	AVNNQFTMGGPAISMASPMSIPTNTMHYGS	Phospho T23 0.03%
20.9	3255.3382	0.0043	AVNNQFTMGGPAISMASPMSIPTNTMHYGS	Phospho S30 0.00%
19.6	3255.3382	0.0043	AVNNQFTMGGPAISMASPMSIPTNTMHYGS	
17.1	3255.3382	0.0043	AVNNQFTMGGPAISMASPMSIPTNTMHYGS	Phospho T25 0.00%
11.9	3255.3382	0.0043	AVNNQFTMGGPAISMASPMSIPTNTMHYGS	Phospho T7 0.00%
11.3	3255.357	-0.0145	GPSSPAHSGALDLGVSRRHQNAMGREKEL	
7.4	3255.3389	0.0036	NLKGQVLSTINTNQMNNSHAVISPCSRF	

Table S2. Related to Figure 2. All Matches Table

Determination of the Carm1 phosphorylation site as shown in Figure 2B. All peptides in the reference proteome matching the mass of the parent ion are listed, along with the corresponding Mascot Score (and phosphorylation site localization probabilities, where applicable) for each possible interpretation.

siRNA Targeting Sequences (Figures 3, 4, 7)	
<i>Mapk12</i> (p38 γ) Dharmacon (Cat# L-062913-00)	AAUGGAAGCGUGUGACUUA CGACUCCUUUGAUGAUGUA
<i>Mapk14</i> (p38 α) Dharmacon (Cat# L-040125-00)	GGGAGGUGCCCGAACGAUA GCAAGAAACUACAUUCAGU ACAUUCGGCUGACAUAUU GAAAUGGAGUCCUGAGCAC
<i>Carm1</i> Dharmacon (Cat# L-048766-00)	GCUACAUGCUCUCAAUGA GGAAGCACCUAUAAUCUCA
<i>Sntb1</i> (β 1-Syn) Dharmacon (Cat# L-049361-00)	GGCAUAAAGUUCUGGUGAA CCACUGGACAAGAAGCAUA GAAUCAGGAUGCUCUAUUU GAAUGCCGCUUGACCAUAC
ON-TARGETplus Non-targeting siRNA #1 Dharmacon (Cat# D-001810-01)	UGGUUUACAUGUCGACUAA

Table S3. Related to STAR Methods. siRNA Targeting Sequences

For ChIP (Figure 7)	
<i>Myf5</i> TSS Fwd	TAATAACGCCAGCTACAGG
<i>Myf5</i> TSS Rev	TTCAGCAGAGAGGGAGACAC
<i>Myf5</i> +0.5kb Fwd	CAAAGCTTGCAAGAGGAA
<i>Myf5</i> +0.5kb Rev	CTGGAGGCTCTCAATGTAGC
<i>Myf5</i> +0.7kb Fwd	GAGGGAACAGGTGGAGAACT
<i>Myf5</i> +0.7kb Rev	CAATGGTCCCCTGGTTTAG
<i>Myf5</i> +1.5 kb Fwd	ACAGCAGCTTTGACAGCATC
<i>Myf5</i> +1.5 kb Rev	GGACAGACTGCCATGACTGA
β -globin (<i>Hbb</i>) TSS Fwd	CAGGGAGAAATATGCTTGTCATCA
β -globin (<i>Hbb</i>) TSS Rev	GTGAGCAGATTGGCCCTTACC
For RT-qPCR (Figure 7 and S7)	
<i>Myf5</i> Fwd	TGACGGCATGCCTGAATGTA
<i>Myf5</i> Rev	ATCTGCAGCACATGCATTTGATA
<i>Rps18</i> Fwd	AACGGTCTAGACAACAAGCTG
<i>Rps18</i> Rev	AGTGGTCTTGGTGTGCTGAC
<i>Pax7</i> Fwd	GACGACGAGGAAGGAGACAA
<i>Pax7</i> Rev	ACATCTGAGCCCTCATCCAG
<i>Myod1</i> Fwd	TGATGGCATGATGGATTACAG
<i>Myod1</i> Rev	GTAGTAGGCGGTGTCGTA
<i>MyoG</i> Fwd	CAGTGAATGCAACTCCCACAG
<i>MyoG</i> Rev	ATGGACGTAAGGGAGTGCAGA
<i>Mapk12</i> Fwd	TGGGCTACTGGATGTGTTCA
<i>Mapk12</i> Rev	CTTCAGCCCCTTCAACATCT
<i>Carm1</i> Fwd	TCCTCATCCAGTTTGCCACAC
<i>Carm1</i> Rev	ATTCCTCTGTCCGCTCACTGA
<i>Crlf1</i> Fwd	GCAGAAGTCACACAAGAC
<i>Crlf1</i> Rev	GGATGGCCTATCCTTAGAG
<i>Pde10a</i> Fwd	CCTGCTATAACCACCTGA
<i>Pde10a</i> Rev	CCTTCTCCCCTGATTGA
<i>Fry</i> Fwd	GAGTGCCTGAAGAACAATGA
<i>Fry</i> Rev	AGAGTCTGGTGACGGAAATA
<i>Fam129a</i> Fwd	GGCCATCGAGAAGGTTAAG
<i>Fam129a</i> Rev	AGTGGGCAGTGTGATTTG
<i>Fst</i> Fwd	CAGTGACAATGCCACATAC
<i>Fst</i> Rev	CCTCTTCCCTCCGTTTCTT
<i>Eps8</i> Fwd	GAACGGAGCACAACTCTTT
<i>Eps8</i> Rev	ACTCGGAGCTTCCACTACT
<i>Dio2</i> Fwd	ACTCGGTCATTCTGCTCAAG
<i>Dio2</i> Rev	GATTCAGGATTGGAGACGTG
For ddPCR (Figures S5)	
<i>Mapk12</i> Fwd	AGAACGTCATTGGGCTACTG
<i>Mapk12</i> Rev	CTGGATTCTGTCTTCACTCAGG
<i>Mapk12</i> Probe	56-FAM/CACAGACTT/ZEN/CTACCTGGTGATGCCAT/3IABkFQ
<i>Tbp</i> Fwd	CGTGAATCTTGGCTGTAACTTG
<i>Tbp</i> Rev	GTCCGTGGCTCTCTTATTCTC
<i>Tbp</i> Probe	5HEX/ATCCCAAGC/ZEN/GATTTGCTGCAGTC/3IABkFQ

Table S4. Related to STAR Methods. Primers Used for ChIP, RT-qPCR, and ddPCR Analyses

Fwd, Forward primer; Rev, Reverse primer