**INVENTORY OF SUPPLEMENTAL MATERIALS**

**Supplemental Figures and Figure Legends**

Figure S1: Related to Figure 1; This figure provides information comparing the protein levels of M&M MEFs transduced with either lenti-MyoD or lenti-Myf5, with endogenous MyoD and Myf5 protein levels in C2C12 cells.

Figure S2: Related to Figure 1; This figure provides the information used to determine that quantities of lenti-MyoD and lenti-Myf5 we selected produced equivalent amounts of protein.

Figure S3: Related to Figure 1; This figure provides information regarding the differentiation of M&M MEFs with increased lenti-Myf5 MOIs and determination of the viral-response genes eliminated from the expression analysis.

Figure S4: Related to Figure 5; This figure provides additional data relating to the chimeric constructs described in Figure 5: Induction of Gal4-Luc by Gal4-fusion constructs in 10T1/2 cells, induction of myogenic genes and differentiation of M&M MEFs by the Myf5 with the MyoD activation domain, induction of myogenic genes by Myf5 containing MyoD's bHLH domain and MyoD lacking its activation domain, and H4Ac ChIPs in M&M MEFs transduced with the Myf5 containing MyoD's bHLH domain and MyoD lacking its activation domain.

Figure S5: Related to Figure 6: Expression of myogenic genes in M&M MEFs transduced with lenti-MyoD or lenti-Myf5 alone or with combinations of lenti-MyoD and lenti-Myf5.

**Supplemental Experimental Procedures**

Determination of Endogenous MyoD and Myf5 levels

Balancing MyoD and Myf5 protein levels

RNA-seq methodology

Native and cross-linked ChIP procedures

ChIP peak calling

Motif analysis

Antibodies

Expression and lentiviral plasmid constructs

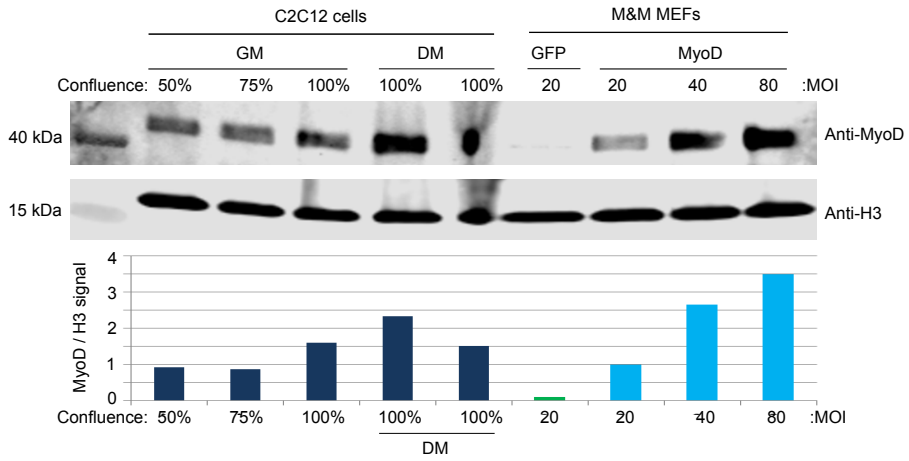
RT-qPCR primers for expression analyses

qPCR primers for ChIP quantification

Oligos for EMSA gel shift assays

Figure S1: Comparison of lenti-MyoD and lenti-Myf5 to endogenous MyoD and Myf5 protein levels. Support for main Fig 1 and main Fig 3.

**A**. Western of MyoD in C2C12s and lenti-transduced M&M MEFs




**B**. qRT-PCR of Myf5 in C2C12s and lenti-transduced M&M MEFs

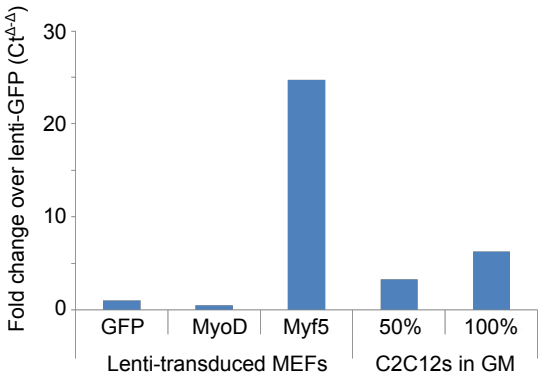
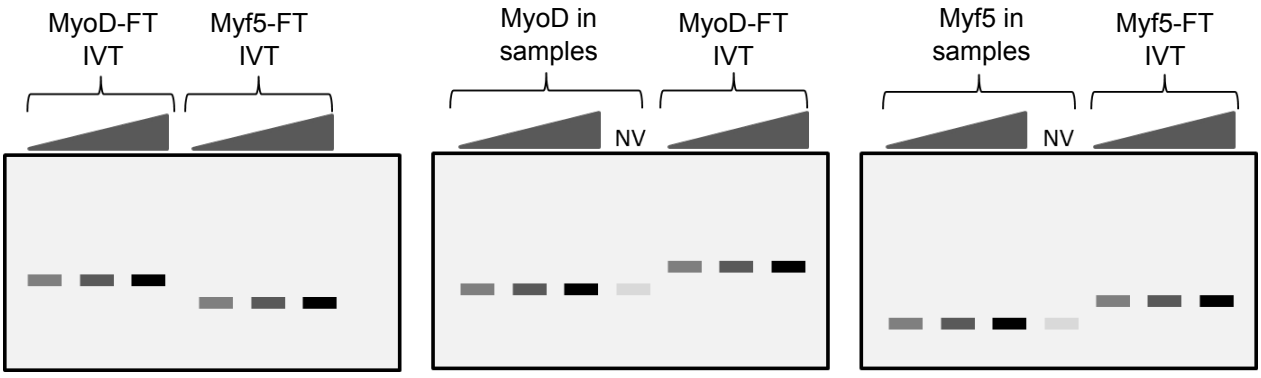


Figure S2: Quantification of lenti-driven Myf5 protein levels compared to lenti-driven MyoD levels
Support for main Fig 1 and main Fig 3

**A.** Schematic for determing the relative abundance of Myf5 and MyoD




Balance standards with anti-Flag

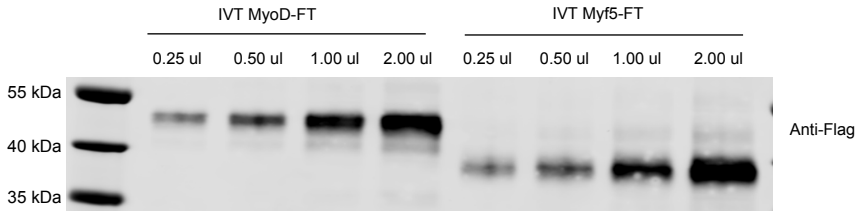
Lenti-MyoD in M&M MEFs compared to the standard with anti-MyoD

Lenti-Myf5 in M&M MEFs compared to the standard with anti-Myf5

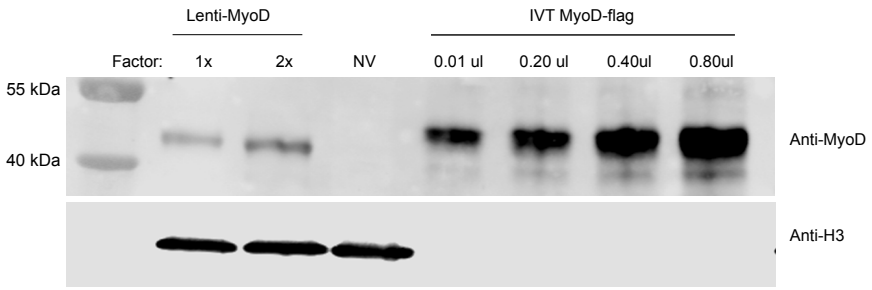
1. Use in-vitro translated (IVT) flag-tagged MyoD and Myf5 to make protein standards and use an anti-Flag Ab to balance the MyoD and Myf5 standards.

2. Compare lysates from M&M MEFs infected with lenti-MyoD or lenti-Myf5 to the standards to identify the amount of lenti-Myf5 that results in the same protein level as 1x MyoD.

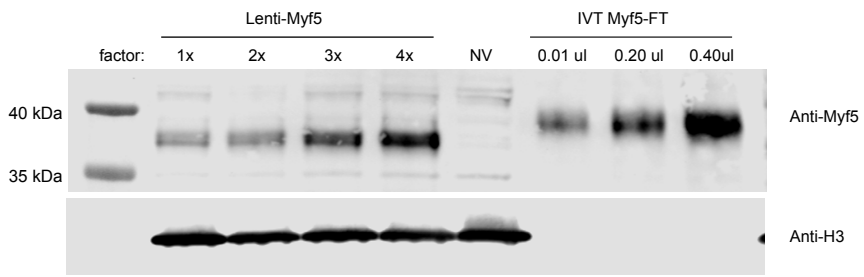
**B.** Quantifying flag-tagged MyoD and Myf5 standards




**C.** Quantifying MyoD




**D.** Quantifying Myf5




E. Average ul standard per 1x MyoD

| | Rep 1 | Rep 2 |
|---|---|---|
| MyoD: | 0.0252 | 0.0253 |
| Myf5: | 0.0287 | 0.0339 |

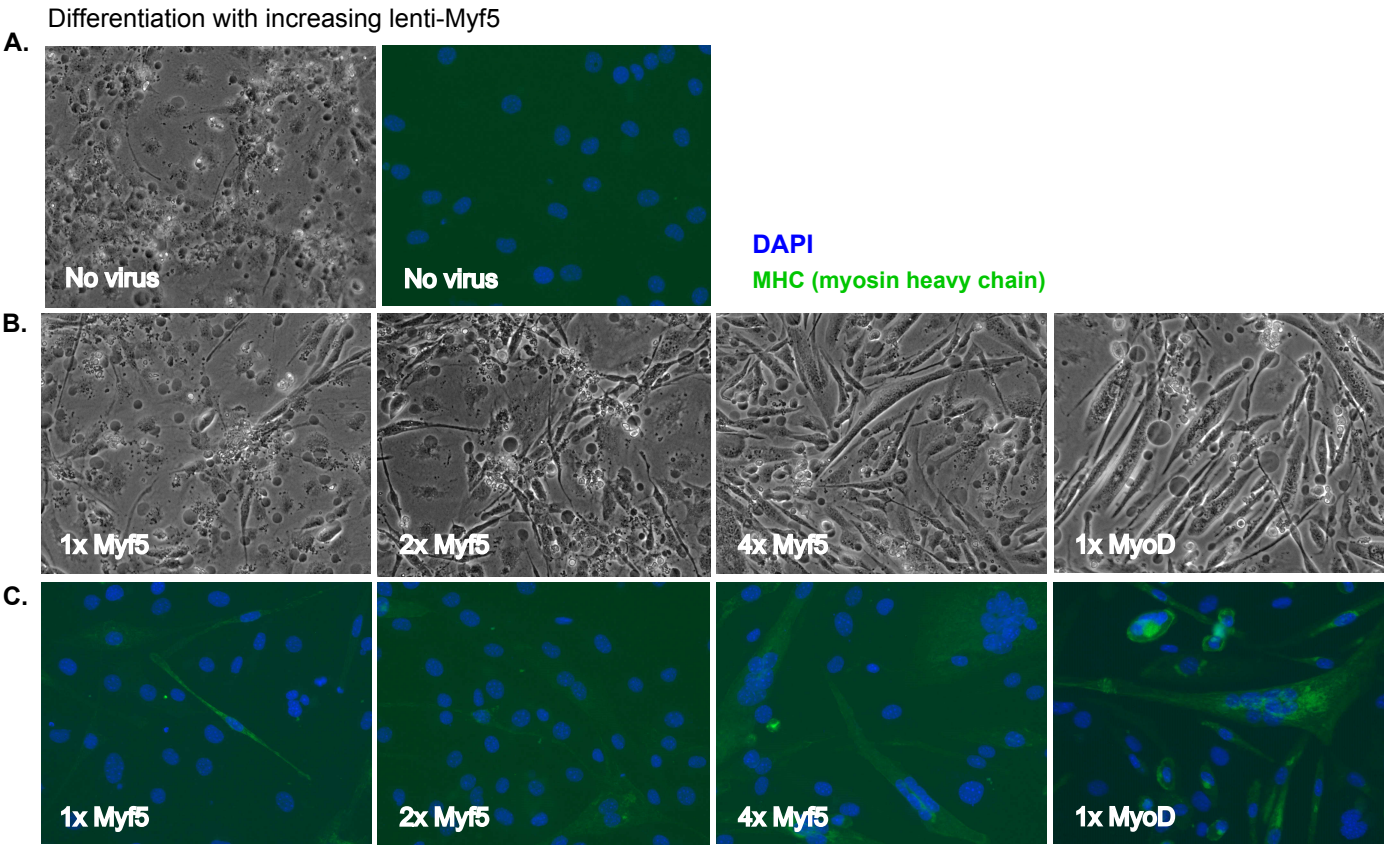
F. Adjust for differences in standards

| | Rep 1 | Rep 2 |
|---|---|---|
| MyoD: | 0.0271 | 0.0272 |
| Myf5: | 0.0287 | 0.0339 |

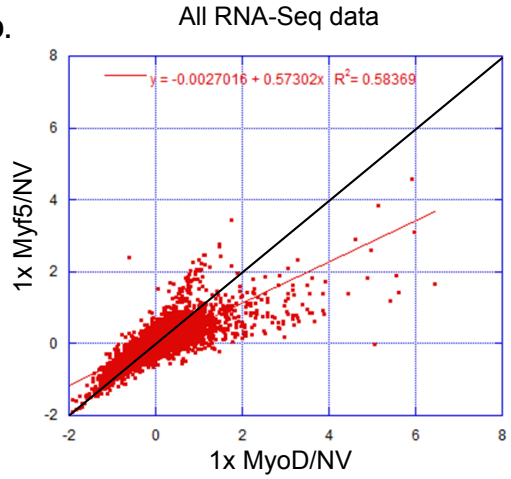
G. Ratio of Myf5 to 1x MyoD

| | Rep 1 | Rep 2 |
|---|---|---|
| MyoD: | 1.0000 | 1.0000 |
| Myf5: | 1.0583 | 1.2458 |

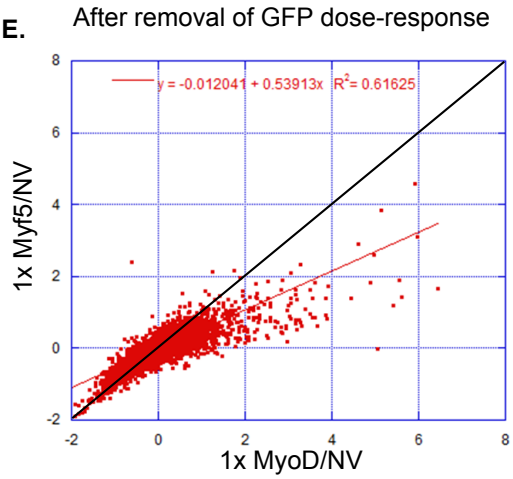
Figure S3: Support for main Fig 1



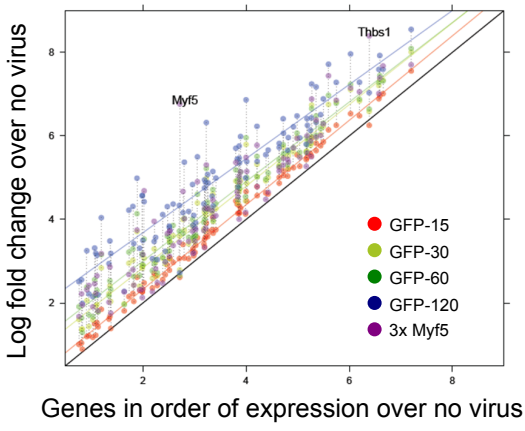

D.




E.




F.




G.

GFP dose-response examples

| | Ifit1 | Lgals3bp |
|---|---|---|
| No virus: | 5.92 | 26.05 |
| GFP-15: | 131.73 | 137.10 |
| GFP-30: | 184.41 | 243.90 |
| GFP-60: | 215.46 | 237.41 |
| GFP-120: | 363.37 | 338.74 |

Figure S4. Support for main Fig 5

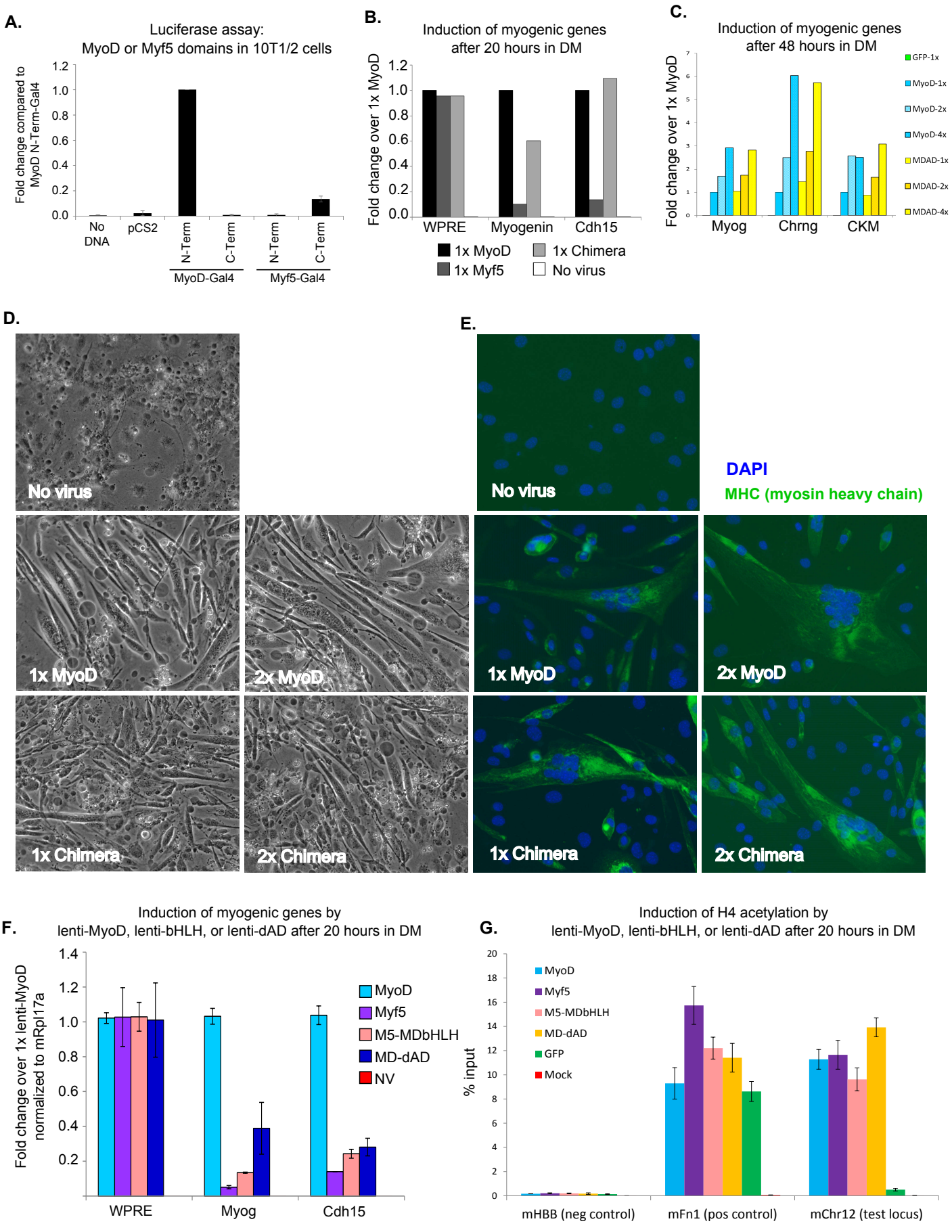
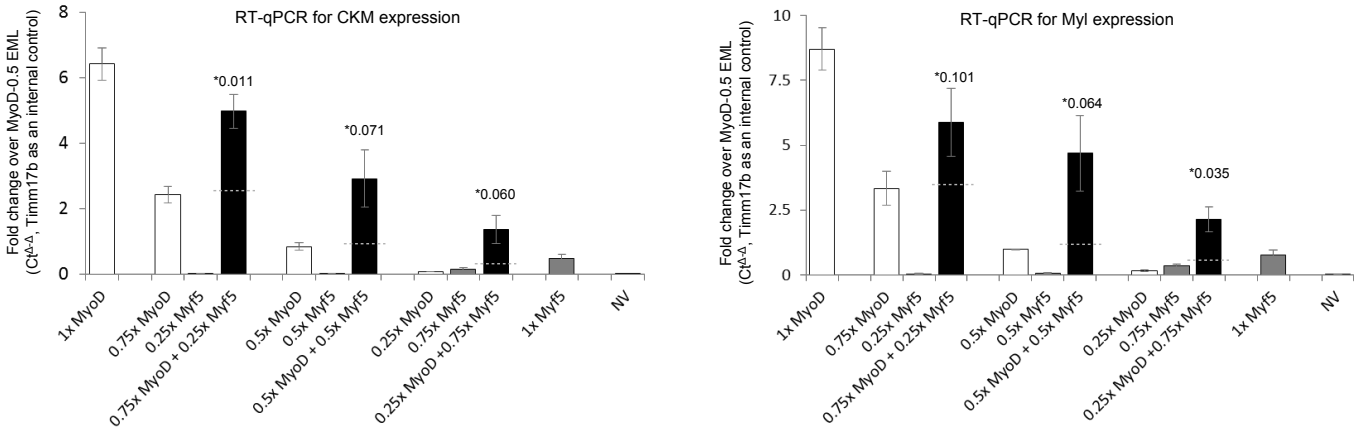


Figure S5. Support for main Fig 1 and 6

**A.**




* pvalue for the comparison between the observed combination of MyoD and the expected sum of the responses to individual factors, computed using a one-sided t-test.

**SUPPLEMENTAL FIGURE LEGENDS**

**Supplemental Figure S1.** Determination of the amount of lenti-MyoD required to match endogenous MyoD (1x). Relates to main Fig 1 and main Fig 3.

(A) Protein was harvested from C2C12 cells in either growth media (GM) or differentiation media (DM) at various densities, as well as M&M MEFs transduced with a range of Lenti-MyoD. Quantitative western blots for MyoD and histone H3 were done using the LiCor Odyssey scanner. Lane one is the ladder. An MOI of 30 was deemed equivalent to the amount of MyoD in the transition between myoblasts and myotubes (between 100% in GM and 100% in DM.) (B) RNA was harvested from C2C12 cycling myoblasts (50% confluent in GM), C2C12 myoblasts just beginning to differentiate (100% in GM), and M&M MEFs transduced with 2x lenti-GFP, 2x lenti-MyoD, or 2x lenti-Myf5. RT-qPCR was done to determine Myf5 level in lenti-transduced cells vs endogenous Myf5 levels in myoblasts. We determined that 2x lenti-Myf5 results in approximately 4 fold more Myf5 mRNA than the highest endogenous level. Thus our 1x Myf5 is likely to be between 1.5-2 fold greater than endogenous Myf5 levels.

**Supplemental Figure S2.** Quantification of lenti-driven Myf5 protein levels compared to lenti-driven MyoD levels. Relates to main Fig 1 and main Fig 3.

To determine that MyoD and Myf5 were expressed at equivalent protein levels, we devised a way to compare them to Flag-tagged standards using quantitative western blots. This approach normalizes for differences in antibody affinity, allowing a direct relationship between MyoD and Myf5 protein levels to be inferred. (A) Schematic for determining the relative abundance of MyoD and Myf5 protein levels. Flag-tagged MyoD and Myf5 were in-vitro translated (MyoD-FT IVT and Myf5-FT IVT) and assayed on the same blot with an anti-Flag antibody. Dilutions of the IVT standards were run to ensure the signal was in a linear range. The standards were then compared to samples from either M&M MEFs infected with lenti-MyoD or lenti-Myf5 using anti-MyoD and anti-Myf5 antibodies respectively. H3 was used to ensure that each lane of the MyoD and Myf5 blots had the same amount of total protein loaded. (B) Western blot of MyoD-FT and Myf5-FT standards probed with the anti-Flag Ab. (C) Western blots of lenti-MyoD samples (1x and 2x expression levels) with the MyoD-FT standards. The blot was cut and probed with anti-MyoD and anti-Histone H3 Abs. Background subtraction was from untransduced M&M MEFs (NV = no virus). (D) Western blots of lenti-Myf5 samples (1x-4x) with the Myf5-FT standards. The blot was cut and probed with anti-MyoD and anti-Histone H3 Abs. Background subtraction was from untransduced M&M MEFs (NV = no virus). (E) Signal from lenti-MyoD and lenti-Myf5 samples was normalized to histone H3, divided by the factor of the virus and then extrapolated onto the standard curves created from the FT-MyoD and FT-Myf5 samples. *(Blots C,D)*. (F) The lenti-MyoD samples were adjusted based the normalization of the FT standards using the anti-Flag blot. *(Blot B).* (G) Ratio of Myf5 protein to MyoD protein.

**Supplemental Figure S3.** Relates to main Fig 1
(A-C) Differentiation of M&M MEFs with a range of lenti-Myf5 titers. Cells were transduced with lenti-MyoD (1x) or lenti-Myf5 (1x, 2, and 4x) for 24 hours, recovered in growth media for an additional 24 hours, and then switched into differentiation media for 72 hours. (A) Bright field and IF on control M&M MEFs (untransduced). (B) Bright field image of cells demonstrate some differentiation of M&M MEFs with 4x lenti-Myf5. (C) IF for MHC on M&M MEFs transduced with lenti-MyoD or lenti-Myf5. At 4x lenti-Myf5 we observe some multinucleated myotubes, though the MHC staining is not a bright as cells transduced with 1x MyoD. (D-G) M&M MEFs were transduced with a range of lenti-GFP titers and expression changes were assayed by RNA-Seq. Genes that displayed an upward trend with increasing lenti-GFP (progressively increased in 3 of the 4 titers) were removed from the analysis. (D-E) Changes in gene expression in response to lenti-MyoD or lenti-Myf5 before and after the GFP-dose responsive genes were removed. (F) Genes initially called as Myf5-specific respond to increasing amounts of GFP. Because we had to use greater volumes of lenti-Myf5 to achieve the same expression as lenti-MyoD it is very likely that all these genes are a response to viral infection rather than Myf5. (G) Examples of normalized read counts in GFP dose response genes. *IFit1* increases with GFP titer while *Lgals3bp* has a slight dip in one sample (highlighted in red).

**Supplemental Figure S4.** Gal4-fusions and domain swaps. Relates to main Fig 5.

(A) 10T½s were transfected with effector plasmids (Gal4 DNA binding domain fused to portions of MyoD or Myf5) and the pGal4 reporter plasmid. Cells were grown for 24 hours after transfection and then assayed as above using the Promega Dual-Luciferase Reporter system. pRL-Renilla was omitted as we discovered that the effector plasmids had differential and unexpected effects on the expression of pRL-Renilla in this cell line under DM conditions. For these luciferase assays we took the ratio of signal for each effector plasmid over the signal from the MyoD-NT-Gal4 effector. We did three sets of assays spread out over three weeks and each assay contained 3 biological replicates. (B) RT-qPCR analysis of gene induction in M&M MEFs by either lenti-MyoD or lenti-Chimera after 48 hours in DM. (C) (D) Phase contrast microscopy of M&M MEFs transduced with either lenti-MyoD or lenti-Chimera and incubated in DM for 72 hours. (E) IF for MHC in M&M MEFs transduced with either lenti-MyoD or lenti-Chimera and incubated in DM for 72 hours. (F) RT-qPCR analysis of gene induction by lenti-MyoD, lenti-Myf5, lenti-M5-MDbHLH, and lenti-MD-dAD in M&M MEFs. (G) ChIP of H4Ac from M&M MEFs transduced with lenti-MyoD, lenti-Myf5, lenti-M5-MDbHLH, lenti-MD-dAD, or lenti-GFP for 24 hours and incubated in DM for 20 hours. The mock IPs replaced the anti-H4Ac primary antibody with normal rabbit serum. Each ChIP was done 3 times and the error bars represent the standard deviation of the ChIPs. The Fribronectin 1 promoter was used as a positive control even though expression and H4Ac are somewhat reduced in lenti-MyoD samples due to differentiation potential. The test locus is a region of Chr12 that contains a MyoD/Myf5 binding site but is not adjacent to any genes and is therefore not impacted by any changes in expression.

**Supplemental Figure S5.** Synergism between MyoD and Myf5 and differentiation potential of Myf5 over expression. Relates to main Fig 1 and main Fig 6.

(A) RT-qPCR analysis of CKM and Myl1. M&M MEFs were transduced with variable amounts of lenti-MyoD and/or Lenti-Myf5 and differentiated for 20 hours in DM. White dashed white lines indicate the expected signal if MyoD and Myf5 acted in an additive manner.

**SUPPLEMENTAL EXPERIMENTAL PROCEDURES**

**Determination of Endogenous MyoD and Myf5 levels:**
MyoD and Myf5 protein and RNA was isolated from M&M MEFs transduced with range of lenti-MyoD or lenti-Myf5 infection titers and compared to protein and RNA isolated from differentiating C2C12 myoblasts. M&M MEFS were transduced in growth media (GM) for 24 hours and nuclear protein was collected after an additional 20 hours in differentiation media (DM). Nuclear protein was collected from C2C12s at the following times: 50% confluence in GM (cycling myoblasts), 75% confluence in GM (myoblasts), 100% confluence in GM (elongating myocytes), 100% confluence in DM for 24 hours (myocyte to myotubes transition), and 100% confluence in DM for 48 hours (myotubes). MyoD levels were assayed by two-color quantitative western blotting where the MyoD signal was normalized to histone H3 for each sample (Fig. S1). M&M MEFs transduced with lenti-MyoD at an MOI of 30 was deemed equivalent to the early stages of myogenic differentiation

**Balancing MyoD and Myf5 protein levels:**
To determine that MyoD and Myf5 were expressed at equivalent protein levels, we devised a way to compare them to Flag-tagged standards using quantitative western blots. This approach normalizes for differences in antibody affinity, allowing a direct relationship between MyoD and Myf5 protein levels to be inferred. Flag-tagged MyoD and Myf5 were in-vitro translated and assayed on the same blot with an anti-Flag antibody. This allowed us to create standards that had equal amounts of MyoD or Myf5 in them. Dilutions of the IVT standards were run to ensure the signals were in a linear range for each antibody. M&M MEFs transduced with a range of MOIs were harvested and anti-H3 was used to normalize for any differences in sample loading. We then compared the signal from our lenti-MyoD or lenti-Myf5 transduced cells to the flag-tagged standards using anti-MyoD and anti-Myf5 antibodies respectively.

**RNA-seq:**
Total RNA was extracted from cells using the Qiagen RNeasy kit. Sequencing libraries were prepared from total RNA using the TruSeq RNA Sample Prep Kit (Illumina) according to the manufacturer's instructions, poly(A) selected, and subjected to Illumina sequencing using standard protocols to generate 100 bp paired-end reads. Files were demultiplexed of indexed reads and generated in FASTQ format using Illumina's CASAVA v1.8. software. We removed reads that did not pass Illumina's quality threshold and the remaining reads were mapped to the mouse genome (build MM9).

**xChIP-seq for MyoD and Myf5:**
Cross-linked ChIP was performed as previously described (Cao et al., 2010). Briefly, ~$10^8$ cells were fixed in 1% formaldehyde for 11 minutes, quenched with glycine, lysed, and then sonicated to generate final DNA fragments of 150–600 bp. The soluble chromatin was diluted 1:10 and pre-cleared with protein A:G beads for 2 hours. Remaining chromatin was incubated with primary antibody overnight, then protein A:G beads were added for an additional 2 hours. Beads were washed and then de-crosslinked overnight. ChIP samples were validated by qPCR and then prepared for sequencing per the Nugen Ovation Ultralow library system protocol with direct read barcodes.

**nChIP-seq for H4AC:**
Native ChIP for histone acetylation was performed by combining the nuclei isolation protocol from Gerber et al, 1997 and the MNase digestion and chromatin extraction method from Cao et al, 2010. Briefly: nuclei were harvested from 10 million cells by lysis in RSB+0.1% NP-40 for 10 min. Nuclei were rinsed with RSB and resuspended in 250 µl douncing buffer containing 15 U of MNase, and then

incubated at 37°C for 18 min. The reaction was stopped by adding EDTA to 10 mM and moving the nuclei to ice. 1 ml of hypotonic lysis buffer with protease inhibitors was added to each sample. 50 µl of 0.25 M NaButyrate was added to each sample and the samples were rotated at 4°C for 1 hour. Insoluble material was removed by centrifugation (3800 xg for 5 min, 4°C). 100 µl of the supernatant was removed and reserved for input DNA. 20 µl of antibody was added to 1 ml of soluble chromatin and rotated overnight at 4°C. Nucleosome-antibody complexes were isolated with protein A:G agarose beads in the same manner as the xChIPs. ChIP samples were validated by qPCR and prepared for sequencing per the Nugen Ovation Ultralow library system with direct read barcodes.

**ChIP peak calling:**
We used a peak calling and comparison approach previously used by Fong et al, 2012.
Briefly: Sequences were extracted using the GApipeline software and reads mapping to the X and Y chromosomes were excluded from our analysis. Reads were aligned to the MM9 mouse genome using MAQ and BWA and duplicate sequences were discarded. Each read was extended in the sequencing orientation to a total of 200 bases to infer the coverage at each genomic position. We performed peak calling by an in-house developed R package (peakSig) which models background reads by a negative binomial distribution. We also fit separate model parameters based on the binned GC content of the flanking sequence, which based on our observations, heavily correlates with background read density. Therefore, the significance of the peaks is determined not only by peak height, but also by the GC content of the flanking sequence. We used control ChIP-seq samples to eliminate statistically significant peaks likely due to artifact. We removed all peaks that overlap with the peaks in the control sample at a p-value cutoff of $10^{-5}$, and required all remaining peaks to have a much more significant p-value ($10^{-3}$) than in the control sample

**Motif analysis:**
To find motifs enriched under ChIP-Seq peaks, we selected background sequences using random genomic regions sampled with similar GC content and distance to TSS. The motif z values follow a normal distribution if there is no distinction between foreground and background sequences. To learn a PWM model, we used the output motif instances from the motif discovery tool as the seed to initialize the iterative expectation maximization (EM) refinement process, which is essentially the same as MEME. In some cases, the motifs are extended iteratively as long as there is sequence preference in the flanking region, and refined in the same EM process. Two ChIP-seq replicates were used for both MyoD and Myf5.

**Antibodies:**
Anti-MyoD for western blots (supernatant from hybridoma clone 5.8A harvested in-house); Anti-MyoD for ChIPs (Serum 6975b, a rabbit polyclonal antibody made in-house and previously characterized (Cao et al., 2010; Tapscott et al., 1988); Anti-Myf5 for ChIPs and western blots (Santa Cruz, SC-302x, *lot C0612*); Anti-H4Ac for ChIPs (Active Motif, 39925, multiple lots); Anti-Pol II (N-20) for ChIPs (Santa Cruz, sc-899, *lot C1413*); Anti-Pol II (unphosphorylated CTD, 8WG16) (Abcam, ab817, lot GR153063-402); Anti-Pol II (phosphorylated on CTD Serine 2) (Abcam, ab5095, lot GR124547-2); Anti-histone H3 (Abcam, ab1791, multiple lots); Anti-Flag (Sigma, M2, F 1804); Goat anti-rabbit-800CW (Li-Cor, 926-32211); Goat anti-mouse-800 (Li-Cor, 926-32210); Goat anti-rabbit-680RD (Li-Cor, 926-68071)

**Plasmids:**

Lenti-GFP: pRRLSIN.cPPT.PGK-GFP.WPRE was a gift from Didier Trono (Addgene plasmid # 12252); Lenti-MyoD: pRRLSIN.cPPT.hPGK.MyoD.WPRE. The mMyoD mRNA replaced GFP in addgene plasimid # 12252; Lenti-Myf5: pRRLSIN.cPPT.hPGK.Myf5.WPRE. The mMyf5 ORF replaced GFP in addgene plasimid # 12252; Lenti-Chimera**:** pRRLSIN.cPPT.hPGK.MDAD-Myf5.WPRE. The N-terminus of mMyoD (amino acids 1-51) followed by the first 165 bp of mMyf5 (through the PmlI site) was synthesized by IDT as a g-block containing a BamHI sites on the 5' end. This g-block was then cloned into the BamHI and PmlI sites in pRRLSIN.cPPT.hPGK.Myf5.WPRE. (MDAD= MyoD Activation Domain); pCS2-MyoD; pCS2-MyoD-FT: 3x flag tag added to the 3' end of MyoD; pCS2-Myf5; pCS2-Myf5-FT: 3x flag tag added to the 3' end of Myf5; pCS2-Chimera: pCS2-MDAD-Myf5. The MDAD-Myf5 fusion is from pRRLSIN.cPPT.hPGK.MDAD-Myf5.WPRE; pGL-GG: Promega's pGL3-promoter with the addition of 2 GG E-boxes spaced 18 bp apart; pGL-GC: Promega's pGL3-promoter with the addition of 2 GC E-boxes spaced 18 bp apart. Lenti-bHLH**:** pRRLSIN.cPPT.hPGK.M5-MDbHLH.WPRE. A g-block was designed that contained the bHLH domain of MyoD surrounded by Myf5 flanking sequence. An internal PstI site was mutated to retain the AA but disable the site. This g-block was cloned into pRRLSIN.cPPT.hPGK.Myf5.WPRE with PstI. Lenti-MD-dAD: pRRLSIN.cPPT.hPGK.MyoD-dAD.WPRE. MyoD lacking AAs 3-56 was cloned from the pEMSV-MyoD-dAD plasmid. pCS2-MD-G4-MD: MyoD with the bHLH domain replaced with the Gal4 DNA binding domain. pCS2-M5-G4-M5: Myf5 with the bHLH domain replaced with the Gal4 DNA binding domain. pCS2-MD-G4: The N-terminus of MyoD (AAs 1-79) fused to the Gal4 DNA binding domain. pCS2-G4-MD: The Gal4 DNA binding domain fused to the C-terminus of MyoD (AAs 162-318). pCS2-M5-G4: The N-terminus of Myf5 (AAs 1-53) fused to the Gal4 DNA binding domain. pCS2-G4-M5: The Gal4 DNA binding domain fused to the C-terminus of Myf5 (AAs 135-255).

**RT-qPCR primers for expression analysis:**

| | |
|---|---|
| mMyog F1 | GTCCCAACCCAGGAGATCATT |
| mMyog R1 | GACGTAAGGGAGTGCAGATTGTG |
| mCdh15 F1 | GGACAGAATGGAGCTCAGCGT |
| mCdh15 R1 | CTTCCCACCAGCCCATCTG |
| mRpl17a F1 | GAAGCTCATCTATGAGAAGGC |
| mRpl17a R1 | AAGACGAAGGAGCTGCAGAAC |
| WPRE F1 | CAATTCCGTGGTGTTGTCGG |
| WPRE R1 | AAGGAAGGTCCGCTGGATTG |
| mMyf5 F | TGAGGGAACAGGTGGAGAAC |
| mMyf5 R | AGCTGGACACGGAGCTTTTA |

**qPCR primers for ChIP:**

| | |
|---|---|
| mCh7des_F1 | AGCAGTGCAGAACCAAGTCA |
| mCh7des_R1 | GGGCTCTAAGCATCGAAGGA |
| mMgn 5'_F1 | AACAAGCCTTTTCCGACCTGA |
| mMgn 5'_R1 | TAGAAGTGGGGCTCCTGGTA |
| mMgn 3'_F1 | GAGACATGAGTGCCCTGACC |
| mMgn 3'_R1 | AGGCTTTGGAACCGGATAGC |
| mChrng_5'_F1 | CGCGACTCAGATGTGGTCAA |
| mChrng_5'_R1 | GGTTAGATCCCCAGCACTCG |

| | |
|---|---|
| mChrng_3’_F1 | TGCCCCAACCCCAATACTCT |
| mChrng_3’_R1 | GGGCTGGGATCATGTATGTG |
| mFn1_5’_F1 | GTGTGAGTGCGCGCTTTATC |
| mFn1_5’_R1 | TTAGCGCACTTGAGGGGAAG |
| mCdh15_5'_F1 | CAATGGGTTCTGCTCTGCTC |
| mCdh15_5'_R1 | AGGTTCCCCCAAAGACACTC |
| mHBB_F | CTGCTCACACAGGATAGAGAG |
| mHBB_R | GCAAATGTGAGGAGCAACTGA |
| mChr12_H4Ac-1_F | TCGTCCTGTAGCTCAAAGCG |
| mChr12_H4Ac-1_R | ATCCCTCAAGCATGGCCTTC |

**EMSA oligos:**

| | |
|---|---|
| GS-111 | CAGAGTGACAGGTGTCGGCGGG |
| GS-112 | CCCGCCGACACCTGTCACTCTG |

| | |
|---|---|
| AF252 | AGTCAGGAGCTCAGAGTGACAGGTGGCGGCGGGTCGAGAGTGACAGGTGGCGGCAGATCTAGTCAG |
| AF253 | CTGACTAGATCTGCCGCCACCTGTCACTCTCGACCCGCCGCCACCTGTCACTCTGAGCTCCTGACT |