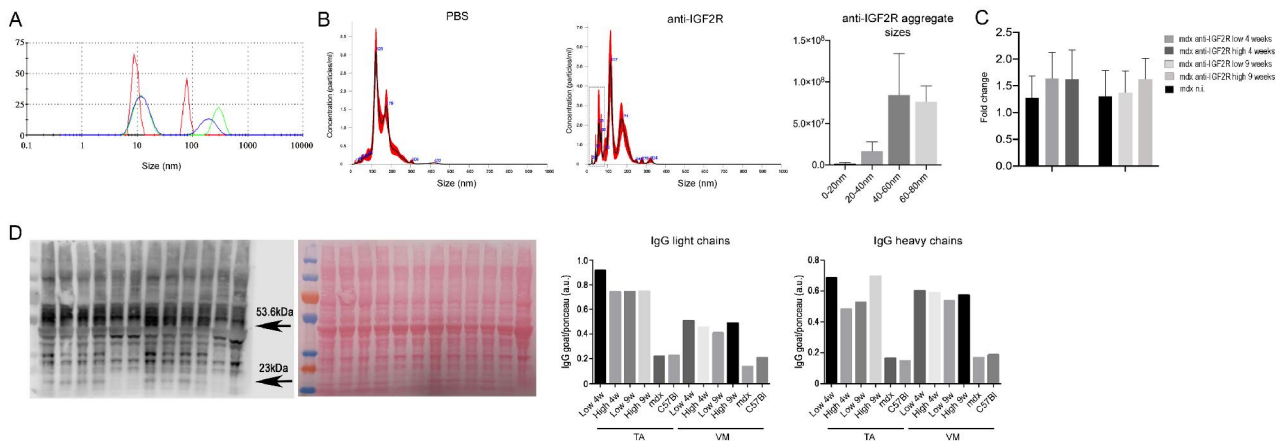


APPENDIX

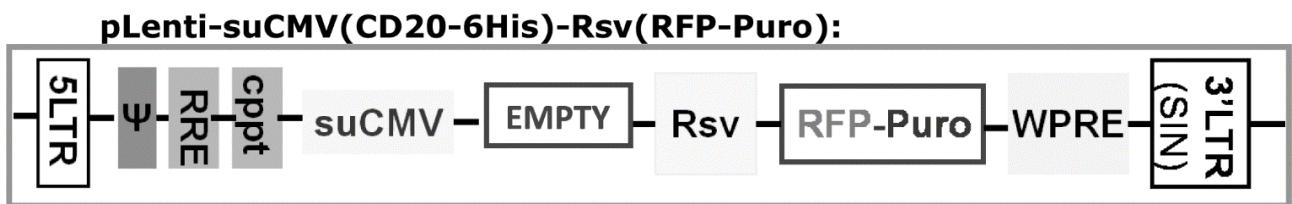
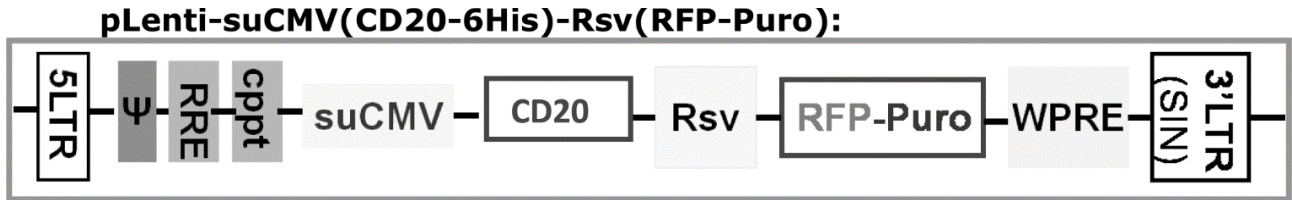
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Appendix Figure S1. (a) Size distribution of anti IGF2R aggregates was detected by DLS technique. N=3 different measurements (red, blue, and green lines) were acquired. The graph shows two major peaks in the ranges of 10-20 nm (centered at 12.8nm \pm 3.77) and 180-200nm (centered at 196.8nm \pm 56), with an intensity percentage of 71.5% and the 28.5%, respectively. PBS1X did not shows notable peaks. (b) NTA detection of anti IGF2R aggregate size distribution. N=5 measurements were acquired and averaged (red lines). Since PBS 1X, used as diluent, presents barely detectable peaks below 100nm, the graph region showing peaks between 0 and 80 nm is likely owned only to IGF2R formulation. Histogram of anti IGF2R particle concentration in the main regions between 0 and 80 nm (One-way ANOVA, $p > 0.1$). (c) Analysis of reactivity against polyclonal goat anti IGF2R antibody in plasma samples collected from high and low dose injected mice treated for 4 and 9 weeks. Reactivity was expressed as fold change over the untreated (n.i.) mouse values. Not significant differences were measured between the samples (One-way ANOVA, $p > 0.05$). (d) Detection of light (23 kDa) and heavy (53.6 kDa) chain goat IgG presence in TAs and VMs from high and low dose anti-IGF2R injected mice. Untreated mdx and C57Bl murine muscles were used as controls. Ponceau staining was used for WB lane quantification.



Appendix Figure S2. Diagram representing the lentiviral vectors with the RFP-Puromycin reporter gene expressed under a Rous sarcoma virus [Rsv] promoter



Appendix Table S1. Primer sequence for RT-qPCR

Gene	Forward (5'-3')	Reverse (5'-3')
β -actin	TGGCACCACACCTTCTACAATGAG	CCGTGGTGGTGAAGCTGTAGCC
IGF2	TCTCGAGGCACCCTAAATTACC	TCAGCAAATGCCCTGAAAG
IGF1	AGAAGTGTGAGTCTCTGTTCC	GCCAGCCTCTAAAAGATTCTG
IGF1R β	ACACACACACACACACAC	CAGACAACCTCCAGCTTTCC

Appendix Table S1. Exact p-values

Figure		Exact p value	Statistical test
1	A	Ratio IGF2/ β -actin: C2C12+IGF1/C2C12:****<0.0001 C2C12 OECD20+IGF1/C2C12:****<0.0001 C2C12 OECD20+IGF1/C2C12+IGF1:****<0.0001	One-way ANOVA
	C	CD20 immunoprecipitation using anti-pSer+pThr in C2C12 cells treated with IGF1 1nM ON/treated with IGF1 1nM 2h: *= 0.03205 CD20 immunoprecipitation using anti-pSer+pThr in C2C12 cells treated with IGF1 1nM ON/untreated C2C12: *= 0.03741 CD20 immunoprecipitation using anti-pSer+pThr in C2C12 cells treated with IGF1 10nM nM 2h/untreated C2C12: **= 0.0046	Two-way ANOVA
	D	CD20 immunoprecipitation using anti-pSer+pThr in ShCTR-treated C2C12 cells treated with IGF1 10nM and anti-IGF2R/treated with IGF1 10 nM: ****<0.0001 CD20 immunoprecipitation using anti-pSer+pThr in ShCTR-treated C2C12 cells treated with IGF1 10nM and anti-IGF2R/treated with anti-FLAG: ****<0.0001 CD20 immunoprecipitation using anti-pSer+pThr in ShCTR-treated C2C12 cells treated with IGF1 10nM and anti-IGF2R/ShCD20-treated C2C12 cells treated with IGF1 10nM and anti-IGF2R: *= 0.0412 CD20 immunoprecipitation using anti-pSer+pThr in ShCD20-treated C2C12 cells treated with IGF1 10nM and anti-IGF2R/treated with IGF1 10 nM: ****<0.0001 CD20 immunoprecipitation using anti-pSer+pThr in ShCD20-treated C2C12 cells treated with IGF1 10nM and anti-IGF2R/treated with anti-FLAG: ****<0.0001	Two-way ANOVA
	E	CD20 immunoprecipitation using anti-pSer+pThr in C2C12 treated with IGF1 1nM/untreated C2C12: ****<0.0001 CD20 immunoprecipitation using anti-pSer+pThr in C2C12 treated with IGF1 10nM and IGF2 100ng/ml/untreated C2C12: ****<0.0001 CD20 immunoprecipitation using anti-pSer+pThr in C2C12 treated with IGF1 1nM and IGF2 10ng/ml/untreated C2C12: ****<0.0001	Two-way ANOVA
	F	IGF2R/ β -actin Untreated C2C12/C2C12 + anti-IGF2R: *= 0.0293	Student t-Test
2	G	Fusion index: Untreated C2C12/ShCD20+anti-IGF2R C2C12: ***= 0.00032 C2C12 + anti-IGF2R/untreated C2C12: **= 0.0064 C2C12 + anti-IGF2R/ShCD20+anti-IGF2R C2C12: ****<0.0001 HsKM+anti-IGF2R/untreated C2C12: *= 0.0421 HsKM+anti-IGF2R/ ShCD20+anti-IGF2R C2C12: ***= 0.00098	Two-way ANOVA
2	H	Myog/ β -actin C2C12 shCD20+anti-IGF2R 2 days DM/C2C12 untreated 2 days DM: ****<0.0001 C2C12 shCD20+anti-IGF2R 4 days DM/C2C12 untreated 4 days DM: **= 0.0100 C2C12 +anti-IGF2R 2 days DM/C2C12 untreated 2 days DM: **= 0.00841 C2C12 +anti-IGF2R 4 days DM/C2C12 untreated 4 days DM: ****<0.0001 C2C12 +anti-IGF2R 6 days DM/ShCD20-treated C2C12 untreated 6 days DM: *= 0.0193 P.M. HsKM+anti-IGF2R/P.M. C2C12 untreated: **= 0.00651 Myf5/ β -actin C2C12 shCD20+anti-IGF2R 4 days DM/C2C12 untreated 4 days DM: *= 0.0411 P.M. C2C12 +anti-IGF2R/C2C12 untreated: *= 0.0483 P.M. C2C12 +anti-IGF2R/ShCD20-treated C2C12 untreated: **= 0.0099 C2C12 +anti-IGF2R 2 days DM/ ShCD20-treated C2C12 untreated 2 days DM: **= 0.0074 C2C12 +anti-IGF2R 4 days DM/C2C12 untreated 4 days DM: ***= 0.00077 C2C12 +anti-IGF2R 4 days DM/ShCD20-treated C2C12 untreated 4 days DM: ***= 0.00069 MyHC/ β -actin	Two-way ANOVA

		C2C12 +anti-IGF2R 2 days DM/C2C12 untreated 2 days DM: ***= 0.0004 C2C12 +anti-IGF2R 2 days DM/ShCD20-treated C2C12 untreated 2 days DM: ***= 0.00088 C2C12 +anti-IGF2R 4 days DM/C2C12 untreated 4 days DM: ****<0.0001 C2C12 +anti-IGF2R 4 days DM/ShCD20-treated C2C12 untreated 4 days DM: ****<0.0001 C2C12 +anti-IGF2R 6 days DM/C2C12 untreated 6 days DM: *= 0.0311	
3	D	pSer+pThr/CD20 TA mdx/TA C57BL6/J: ****<0.0001 VM mdx/VM C57BL6/J: ****<0.0001	One-way ANOVA
	E	IGF2R/GAPDH TA mdx/TA C57BL6/J: ****<0.0001 TA DMD patients/TA healthy volunteers: ****<0.0001 VM mdx/VM C57BL6/J: ****<0.0001 VM DMD patients/VM healthy volunteers: ***=0.0001	One-way ANOVA
	F	IGF2R fold increase TA mdx/TA C57BL6/J: *=0.0392 TA DMD patients/TA healthy volunteers: ****<0.0001 VM mdx/VM C57BL6/J: ****<0.0001 VM DMD patients/VM healthy volunteers: ***=0.0001	One-way ANOVA
4	B	Regenerating fibers/total fibers VM mdx anti-IGF2R High 9w/untreated mdx: ****<0.0001 VM mdx anti-IGF2R Low 9w/untreated mdx: ****<0.0001 mCad+ cells/section TA mdx+anti-IGF2R High 9w/untreated mdx: ****<0.0001 TA mdx+anti-IGF2R Low 9w/untreated mdx: ****<0.0001 TA mdx+anti-IGF2R High 4w/untreated mdx: ***= 0.00033 TA mdx+anti-IGF2R Low 4w/untreated mdx: **= 0.0096 VM mdx+anti-IGF2R High 9w/untreated mdx: ****<0.0001 VM mdx+anti-IGF2R Low 9w/untreated mdx: ****<0.0001 N=10	One-way ANOVA
4	C	Neonatal MHC/ β -actin TA mdx High 4w/untreated mdx: ****<0.0001 TA mdx Low 4w/untreated mdx: ****<0.0001 TA mdx High 9w/untreated mdx: ****<0.0001 TA mdx Low 9w/untreated mdx: ****<0.0001 VM mdx High 4w/untreated mdx: ****<0.0001 VM mdx Low 4w/untreated mdx: ****<0.0001 VM mdx High 9w/untreated mdx: ****<0.0001 VM mdx Low 9w/untreated mdx: **=0.0073 N=10	One-way ANOVA
4	D	Po/CSA 4 weeks of anti-IGF2R treatment TA C57BL6/J/mdx untreated: **= 0.0065 TA mdx Low dose/mdx untreated: ***= 0.00028 TA mdx High dose/mdx untreated: **= 0.0088 VM C57BL6/J/mdx untreated: *= 0.0433 VM mdx Low dose/mdx untreated: ***= 0.00052 Po/CSA 9 weeks of anti-IGF2R treatment TA C57BL6/J/mdx untreated: **= 0.0084 VM C57BL6/J/mdx untreated: *= 0.0368 VM mdx High dose/mdx untreated: **= 0.0048 N=10	One-way ANOVA
5	A	48 kDa calcineurin A TA mdx +anti-IGF2R High 4w/untreated mdx: *=0.0284 60 kDa calcineurin A	Two-way ANOVA

		<p>TA mdx untreated/mdx +anti-IGF2R High 4w: *=0.0311</p> <p>48 kDa calcineurin A VM mdx +anti-IGF2R High 4w/untreated mdx: ****<0.0001 VM mdx +anti-IGF2R High 9w/untreated mdx: ****<0.0001 VM mdx +anti-IGF2R Low 4w/untreated mdx: ****<0.0001 VM mdx +anti-IGF2R Low 9w/untreated mdx: ****<0.0001 VM mdx untreated/C57BL6/J: ****<0.0001</p> <p>60 kDa calcineurin A VM mdx untreated/mdx +anti-IGF2R High 4w: ****<0.0001 VM mdx untreated/mdx +anti-IGF2R High 9w: ****<0.0001 VM mdx untreated/mdx +anti-IGF2R Low 4w: ****<0.0001 VM mdx untreated/mdx +anti-IGF2R Low 9w: **= 0.0065 VM C57BL6/J/mdx untreated: ****<0.0001</p> <p>N=10</p>	
	B	<p>Ratio pCAMKII/CAMKII</p> <p>TA C57Bl6/J/mdx +anti-IGF2R High 4w: *= 0.04534 TA C57Bl6/J/mdx +anti-IGF2R High 9w: *= 0.03898 TA C57Bl6/J/mdx +anti-IGF2R Low 4w: *= 0.04883 TA C57Bl6/J/mdx +anti-IGF2R Low 9w: *= 0.01084 TA mdx untreated/mdx +anti-IGF2R High 4w: **= 0.01 TA mdx untreated/mdx +anti-IGF2R High 9w: *= 0.0209 TA mdx untreated/mdx +anti-IGF2R Low 4w: **= 0.0095 TA mdx untreated/mdx +anti-IGF2R Low 9w: **= 0.0085</p> <p>VM C57Bl6/J/mdx +anti-IGF2R High 4w: *= 0.0453 VM C57Bl6/J/mdx +anti-IGF2R High 9w: *= 0.0491 VM C57Bl6/J/mdx +anti-IGF2R Low 4w: *= 0.0403 VM C57Bl6/J/mdx +anti-IGF2R Low 9w: *= 0.0394 VM C57Bl6/J/mdx untreated *= 0.0469 VM mdx untreated/mdx +anti-IGF2R Low 9w: *= 0.0108 VM mdx +anti-IGF2R High 9w/ mdx +anti-IGF2R Low 9w: **= 0.0010 VM mdx +anti-IGF2R High 9w/ mdx +anti-IGF2R Low 4w: *= 0.022</p> <p>N=10</p>	Two-way ANOVA
6	A	<p>CD20/vinculin</p> <p>TA mdx + anti-IGF2R Low 4w/C57BL6/J: ****= 0.0002 TA mdx + anti-IGF2R High 4w/C57BL6/J: **= 0.0093 TA mdx + anti-IGF2R Low 9w/C57BL6/J **= 0.0021 TA mdx untreated/C57BL6/J **= 0.0011 TA mdx untreated/ mdx + anti-IGF2R High 9w ****= 0.0010 TA mdx untreated/ mdx + anti-IGF2R Low 9w *=0.027</p> <p>pSer+pThr/CD20</p> <p>TA mdx + anti-IGF2R Low 4w/C57BL6/J: ****<0.0001 TA mdx + anti-IGF2R High 4w/C57BL6/J: ****<0.0001 TA mdx + anti-IGF2R Low 9w/C57BL6/J *= 0.0465 TA mdx + anti-IGF2R High 9w/C57BL6/J: **= 0.0054 TA mdx untreated/C57BL6/J **= 0.0018</p> <p>CD20/vinculin</p> <p>VM mdx + anti-IGF2R Low 4w/C57BL6/J: **=0.0039 VM mdx + anti-IGF2R High 4w/C57BL6/J: ****<0.0001 VM mdx + anti-IGF2R Low 9w/C57BL6/J ****<0.0001 VM mdx + anti-IGF2R High 9w/C57BL6/J: ****= 0.0008 VM mdx untreated/C57BL6/J ****<0.0001</p> <p>pSer+pThr/CD20</p> <p>VM mdx + anti-IGF2R Low 4w/C57BL6/J: ****<0.0001 VM mdx + anti-IGF2R High 4w/C57BL6/J: ****<0.0001 VM mdx + anti-IGF2R Low 9w/C57BL6/J ****=0.0002 VM mdx + anti-IGF2R High 9w/C57BL6/J: ****<0.0001</p>	One-way ANOVA

		VM C57Bl6/J/mdx untreated: ****<0.0001 N=10	
6	B	pIGF1R/IGF1R TA mdx untreated/C57BL6/J: ****<0.0001 TA C57Bl6/J/mdx +anti-IGF2R High 4w: ****<0.0001 TA C57Bl6/J/mdx +anti-IGF2R High 9w: ****<0.0001 TA C57Bl6/J/mdx +anti-IGF2R Low 9w: ****<0.0001 VM mdx untreated/C57BL6/J: ****<0.0001 VM C57Bl6/J/mdx +anti-IGF2R High 4w: ****<0.0001 VM C57Bl6/J/mdx +anti-IGF2R High 9w: ****<0.0001 VM C57Bl6/J/mdx +anti-IGF2R Low 4w: ****<0.0001 VM C57Bl6/J/mdx +anti-IGF2R Low 9w: ****<0.0001 N=10	One-way ANOVA
7	C	% CD146/image TA mdx + anti-IGF2R Low 4w/mdx untreated: ****<0.0001 TA mdx + anti-IGF2R High 4w/mdx untreated: ****<0.0001 TA mdx + anti-IGF2R High 9w/mdx untreated: ****<0.0001 % NG2/image TA mdx + anti-IGF2R Low 4w/mdx untreated: ****<0.0001 TA mdx + anti-IGF2R High 4w/mdx untreated: ***= 0.00025 TA mdx + anti-IGF2R High 9w/mdx untreated: ****<0.0001 N=10	One-way ANOVA
7	D	CD146/ β -actin TA mdx + anti-IGF2R High 4w/mdx untreated: ****<0.0001 TA mdx + anti-IGF2R High 9w/mdx untreated: **= 0.0053 TA mdx + anti-IGF2R Low 9w/mdx untreated: ***= 0.00069 NG2/ β -actin TA mdx + anti-IGF2R High 4w/mdx untreated: *= 0.0415 TA mdx + anti-IGF2R High 9w/mdx untreated: *= 0.0236 TA mdx + anti-IGF2R Low 9w/mdx untreated: ***= 0.00065 CD146/ β -actin VM mdx +anti-IGF2R High 4w/untreated mdx: ****<0.0001 VM mdx +anti-IGF2R High 9w/untreated mdx: ****<0.0001 VM mdx +anti-IGF2R Low 4w/untreated mdx: ***= 0.00079 VM mdx +anti-IGF2R Low 9w/untreated mdx: ****<0.0001 NG2/ β -actin VM mdx +anti-IGF2R High 4w/untreated mdx: ****<0.0001 VM mdx +anti-IGF2R High 9w/untreated mdx: ****<0.0001 VM mdx +anti-IGF2R Low 4w/untreated mdx: ***= 0.00055 VM mdx +anti-IGF2R Low 9w/untreated mdx: ****<0.0001 N=10	One-way ANOVA
EV1	A	CD20/ β -actin Untreated/ShCD20: ****<0.0001 OE CD20/Untreated: ****<0.0001 OE CD20/ShCD20: ****<0.0001 N=5	One-way ANOVA
EV2	B	p-CamKII/CamKII ShCD20 + anti-IGF2R C2C12 2 days DM/untreated C2C12 2 days DM: ****<0.0001 ShCD20 + anti-IGF2R C2C12 2 days DM/untreated C2C12 2 days DM: ***=0.0002 P.M. anti-IGF2R C2C12/untreated C2C12: ***= 0.00016 anti-IGF2R C2C12 2 days DM/ C2C12 2 days DM: ****<0.0001	Two-way ANOVA

		anti-IGF2R C2C12 4 days DM/ C2C12 4 days DM: ****<0.0001 anti-IGF2R C2C12 6 days DM/ C2C12 6 days DM: ****<0.0001 P.M. anti-IGF2R C2C12 /untreated Sh-CD20 C2C12: ***= 0.0004 anti-IGF2R C2C12 2 days DM/ Sh-CD20 C2C12 2 days DM: ****<0.0001 anti-IGF2R C2C12 4 days DM/ Sh-CD20 C2C12 4 days DM: ****<0.0001 anti-IGF2R C2C12 6 days DM/ Sh-CD20 C2C12 6 days DM: ****<0.0001	
EV2	C	CalcA/vinculin ShCD20 + anti-IGF2R C2C12 2 days DM/untreated C2C12 2 days DM: **= 0.0034 ShCD20 + anti-IGF2R C2C12 4 days DM/untreated C2C12 4 days DM: ****<0.0001 ShCD20 + anti-IGF2R C2C12 6 days DM/untreated C2C12 6 days DM: **= 0.0058 anti-IGF2R C2C12 4 days DM/ C2C12 4 days DM: ****<0.0001 anti-IGF2R C2C12 6 days DM/ C2C12 6 days DM: *= 0.0102 anti-IGF2R C2C12 4 days DM/ Sh-CD20 C2C12 4 days DM: ****<0.0001 anti-IGF2R C2C12 6 days DM/ Sh-CD20 C2C12 6 days DM: *= 0.0299	Two-way ANOVA
EV2	E	Cyt/Nuc Untreated/S.D. + Ca ²⁺ 5mM: **= 0.0072 Untreated/S.D. + anti-IGF2R + Ca ²⁺ 5mM: ****<0.0001 S.D. + Ca ²⁺ 5mM/S.D. + anti-IGF2R + Ca ²⁺ 5mM: **= 0.0090 N=3	One-way ANOVA
EV2	F	Myod/vinculin S.D. + anti-IGF2R + Ca ²⁺ 5mM/S.D. + Ca ²⁺ 5mM: ****= 0.00044 S.D. + anti-IGF2R + Ca ²⁺ 5mM/anti-IGF2R + Ca ²⁺ 5mM: ****= 0.00092 S.D. + anti-IGF2R + Ca ²⁺ 5mM/mdx untreated: ****= 0.0006 S.D. + Ca ²⁺ 5mM/anti-IGF2R + Ca ²⁺ 5mM: **= 0.0079 N=3	One-way ANOVA
EV3	B	Fluo4+ (%) C2C12 myoblasts + anti-IGF2R/C2C12: *= 0.0433 Mean Fluo C2C12 myoblasts + anti-IGF2R/C2C12: *= 0.035	One-way ANOVA
	E	Fluo4+ (%) C2C12 myoblasts-ShCD20/C2C12: *= 0.0317 C2C12 myoblasts-ShCD20 + anti-IGF2R/C2C12: **= 0.0087	One-way ANOVA
	G	C2C12 anti-IGF2R/C2C12: *= 0.0410	One-way ANOVA
	L	ORAI-1 Untreated/S.D. + Ca ²⁺ 5mM: **= 0.0057 Untreated/S.D. + anti-IGF2R + Ca ²⁺ 5mM: ****= 0.00063 S.D. + Ca ²⁺ 5mM/S.D. + anti-IGF2R + Ca ²⁺ 5mM: **= 0.0074	One-way ANOVA
EV4	B	Ratio pSer-pThr/CD20 S.D. C2C12 myoblasts/untreated C2C12 myoblasts: ****<0.0001 S.D. C2C12 myoblasts/S.D. C2C12 ShC20 myoblasts: ****<0.0001 S.D. + anti-IGF2R + C2C12 myoblasts/untreated C2C12 myoblasts: ****<0.0001 S.D. + anti-IGF2R + C2C12 myoblasts/S.D. + anti-IGF2R + C2C12 ShC20 myoblasts: ****<0.0001 C2C12 ShCD20 myoblasts/S.D. + anti-IGF2R + C2C12 ShC20 myoblasts: ****<0.0001 C2C12 ShCD20 myoblasts/untreated C2C12 myoblasts: ****= 0.000687 Untreated C2C12 myoblasts/C2C12 myoblasts+anti-IGF2R: *= 0.0323	One-way ANOVA