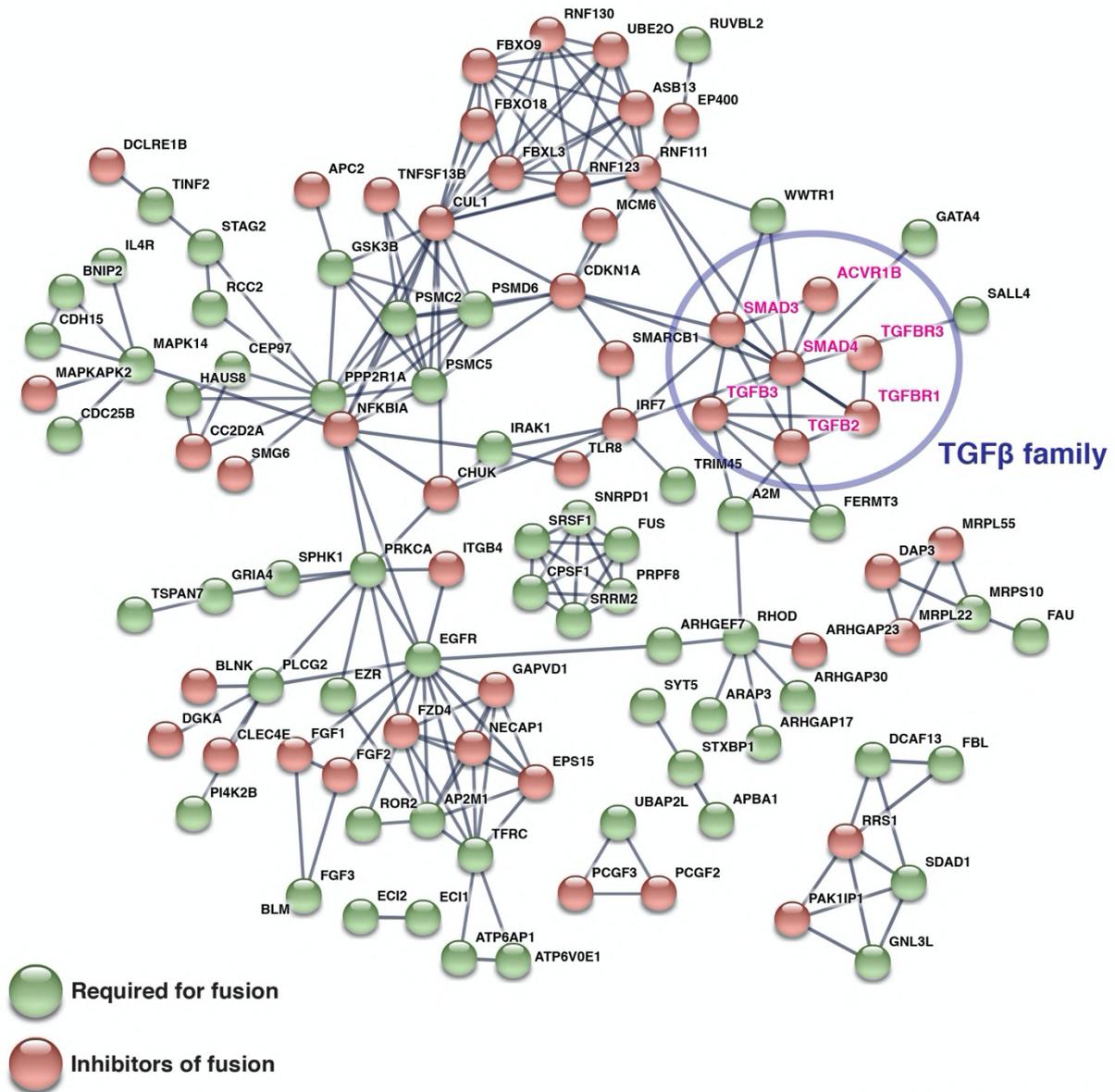


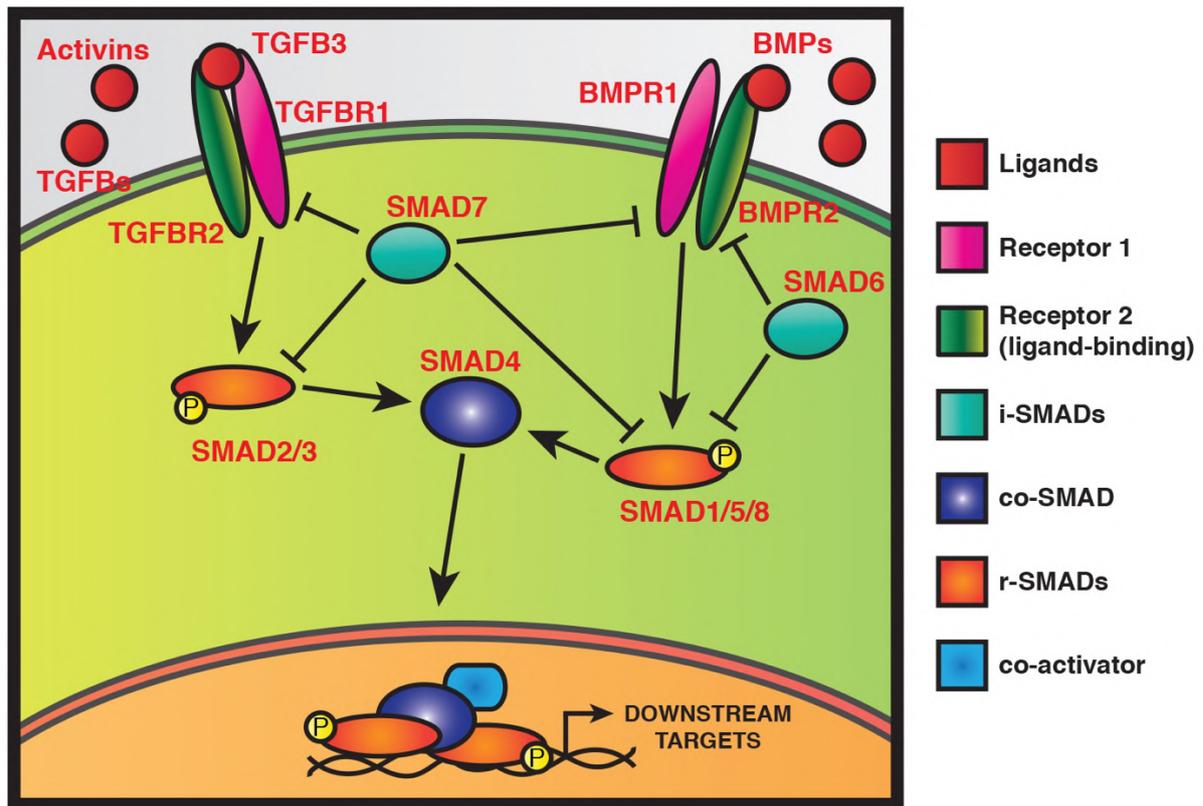
TGFβ signalling acts as a molecular brake of myoblast fusion

SUPPLEMENTARY INFORMATION



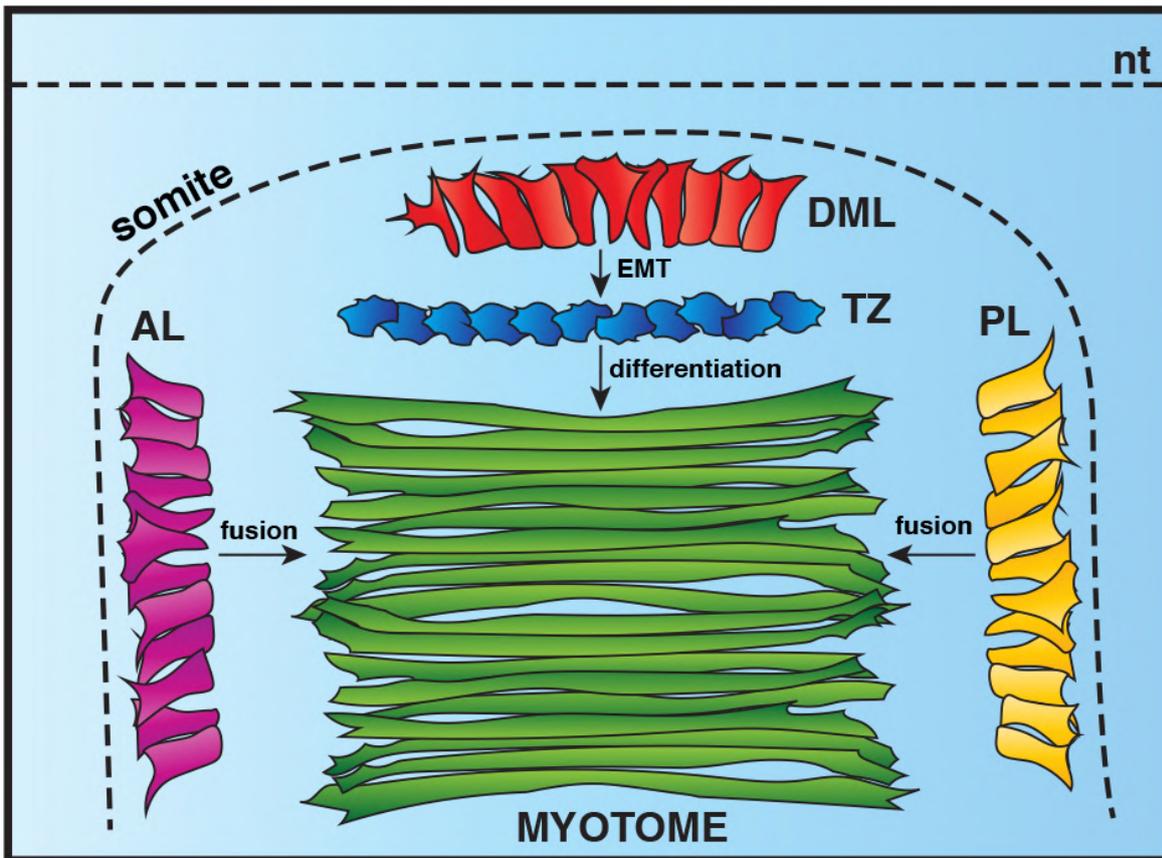
Supplementary Fig. 1

Representation of the predicted protein-protein interactions between candidate genes inhibitory (in red) and necessary (in green) for C2C12 myoblast fusion. The 200 most significant genes for each category were analysed through String software⁴⁰, using highest confidence interaction score. Disconnected nodes were hidden from final graph. Fusion-inhibitory and -necessary molecules were pseudo-coloured manually using Adobe Illustrator. Statistical overrepresentation tests (PANTHER and INGENUITY) on Gene Ontology molecular functions identified the TGFβ family as overrepresented (circled).



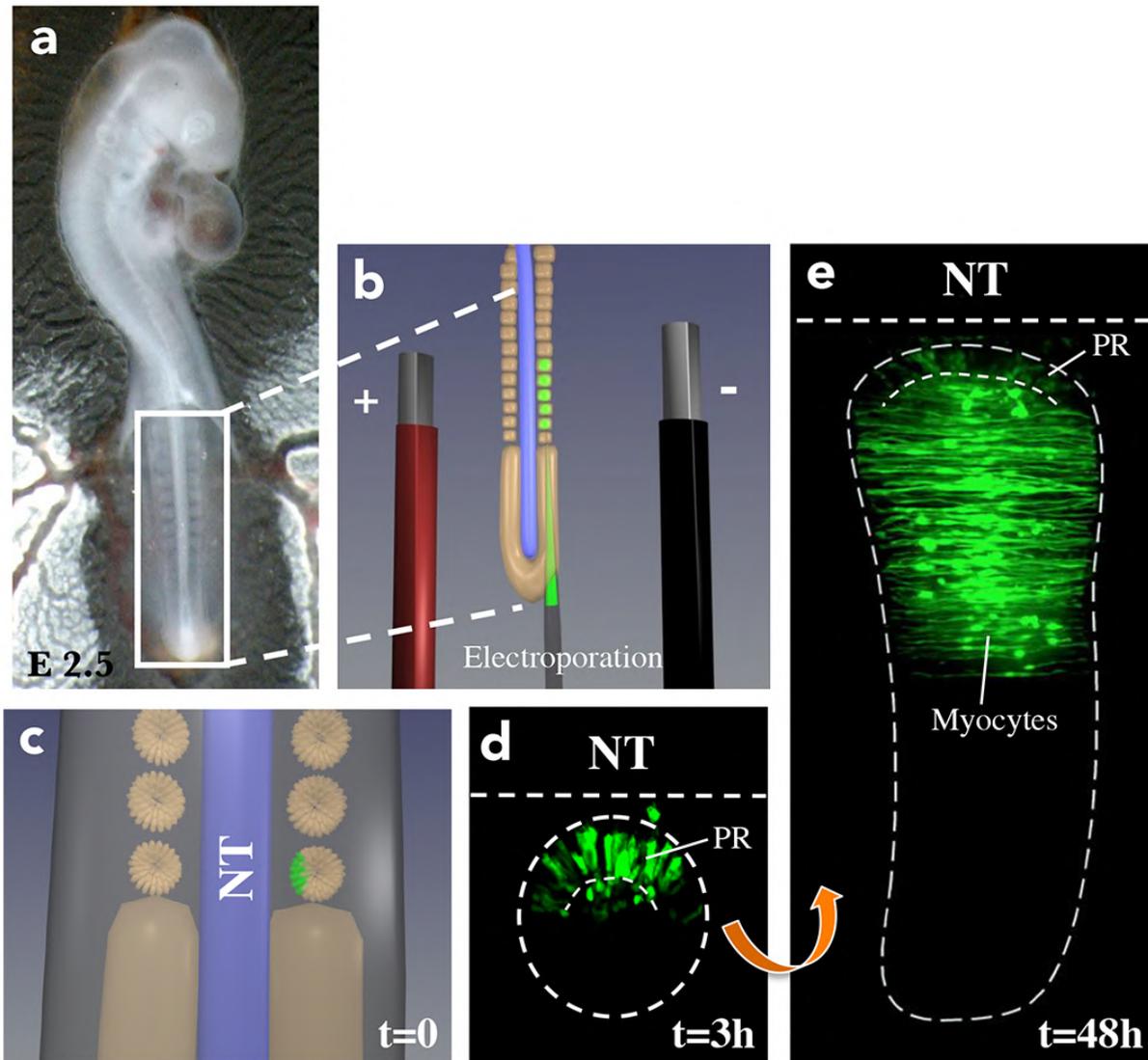
Supplementary Fig. 2

Diagram depicting essential molecules involved in canonical TGF β signalling. TGF β ligands (BMPs, GDFs, TGF β s, among others) bind type 2 receptors, which in turn recruit a type 1 receptor to form a hetero-tetrameric complex. Ligand-bound receptor 2 phosphorylates the serine residues of the receptor 1, which in turn phosphorylates the effector proteins R-SMADs (Receptor-regulated SMADs). BMP receptors only activate SMADs 1/5/8, while TGF β s, Activins and GDFs act through SMADs 2/3. Phosphorylated R-SMADs have a high affinity to SMAD4. The SMAD complex enters the nucleus and binds co-factors and transcription promoters to induce DNA transcription. I-SMADs (Inhibitory SMADs) are involved in negative feedback. SMAD7 competes for receptor 1 binding sites and prevents phosphorylation. SMAD6 competes with SMAD4 exclusively through the BMP SMAD1/5/8 interactions. Diagram has been simplified for clarity and relevance.



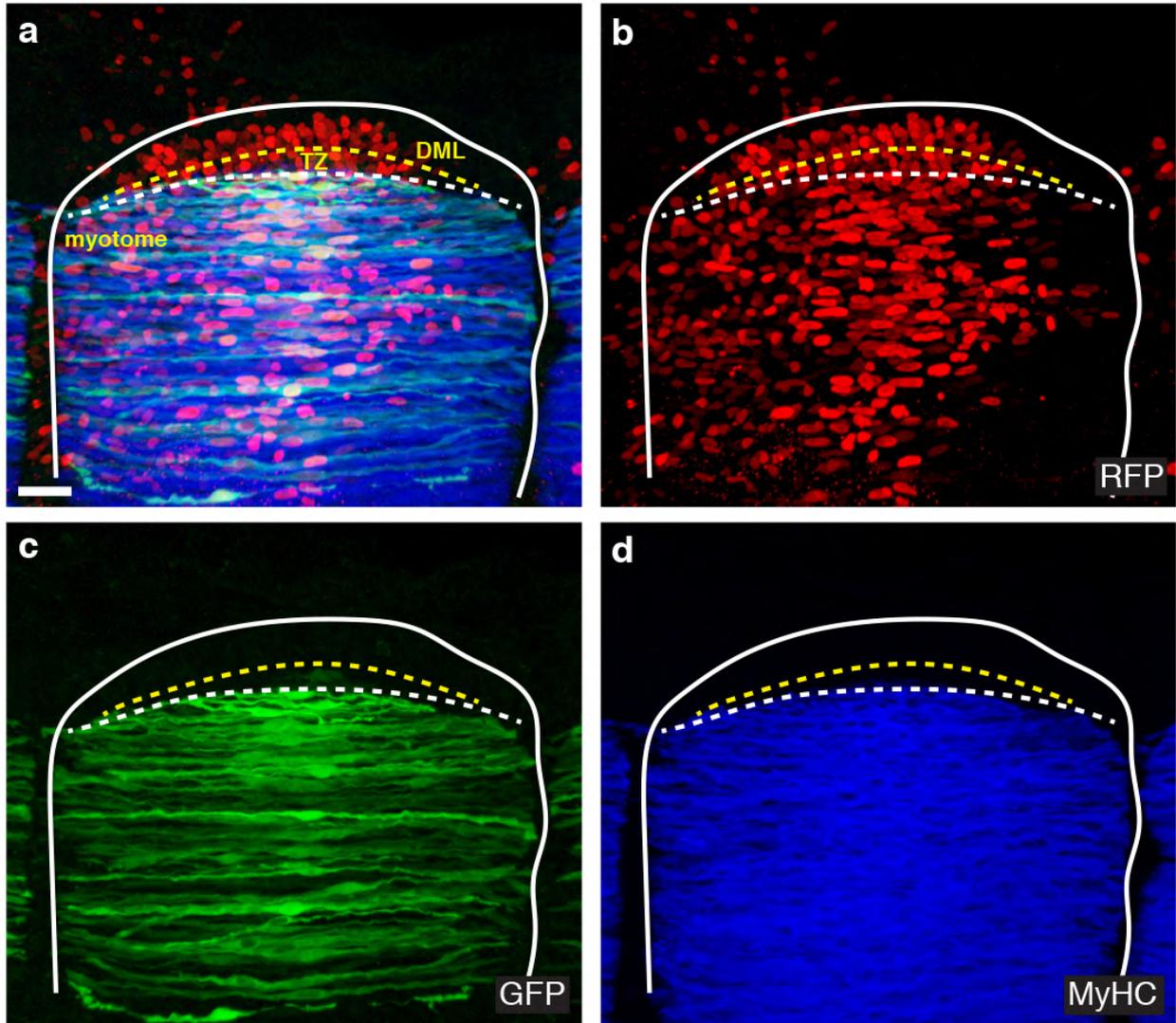
Supplementary Fig. 3

Diagram depicting the somitic regions taking part in the formation of the myotome and the fusion of myocytes. Selected epithelial cells originating from the medial border of the dermomyotome (DML) undergo an epithelial to mesenchyme transition (EMT) that allows their translocation in a region located beneath the dermomyotome, the transition zone (TZ), where they orient in the antero-posterior axis of the embryo²³⁻²⁵. The EMT triggers the entry of cells derived from the DML into the myogenic program. Terminal myogenic differentiation (e.g. MyHC expression) is observed when cells attach to the anterior and posterior borders of somites, at which time they are named myocytes (green elongated fibres). Fusion of myocytes is observed about 24 hours after myotome formation was initiated. Progenitors from the anterior (AL) and posterior (PL) border of the dermomyotome translocate in the myotome where they fuse to existing myocytes⁹. nt: neural tube.



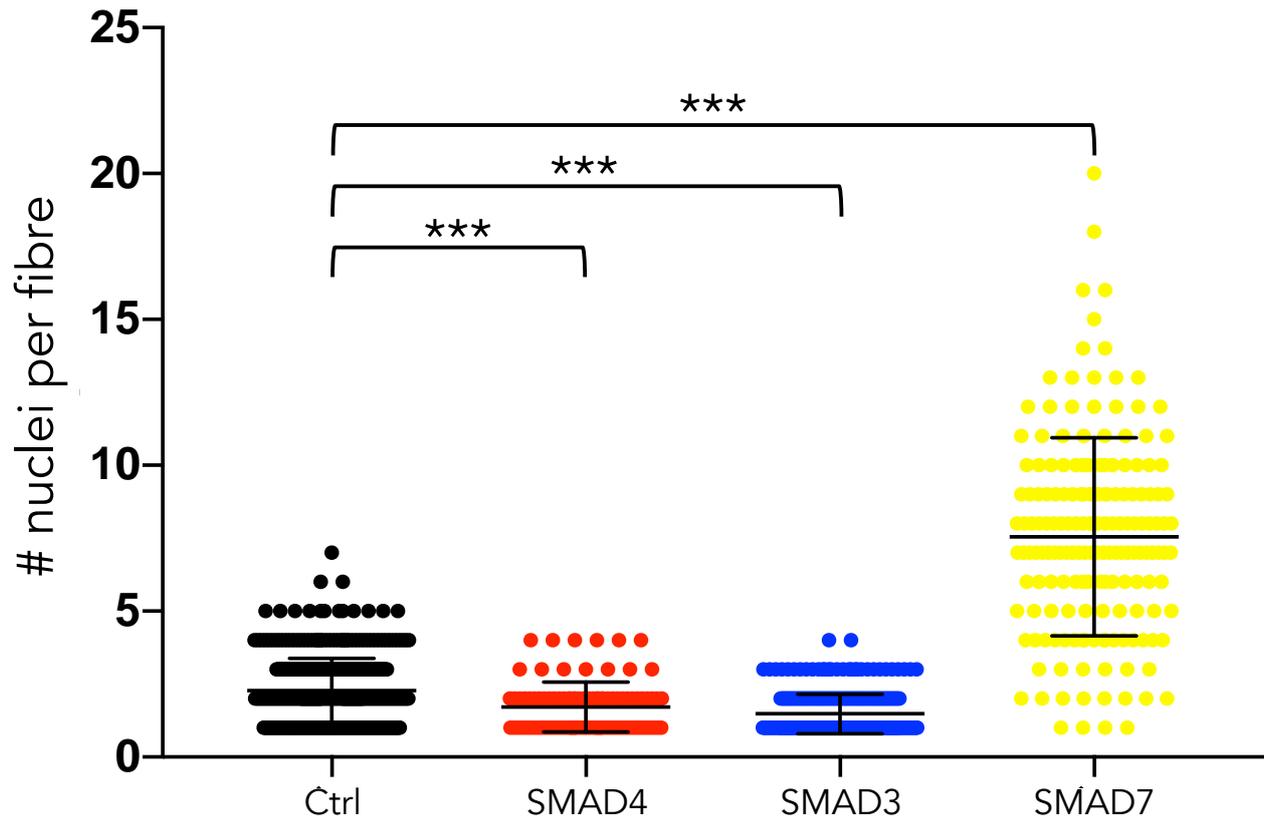
Supplementary Fig. 4

Electroporation protocol used throughout this study. Chicken embryos at 2.5 days of incubation (a) were electroporated in the medial border of newly formed somites (b,c). **d**, shows the epithelial progenitors (PR) expressing a GFP reporter driven by a CAGGS promoter in as short as 3 hours. **e**, Dorsal view of a somite, showing the myocytes that were generated during the two-day incubation time. NT, neural tube.



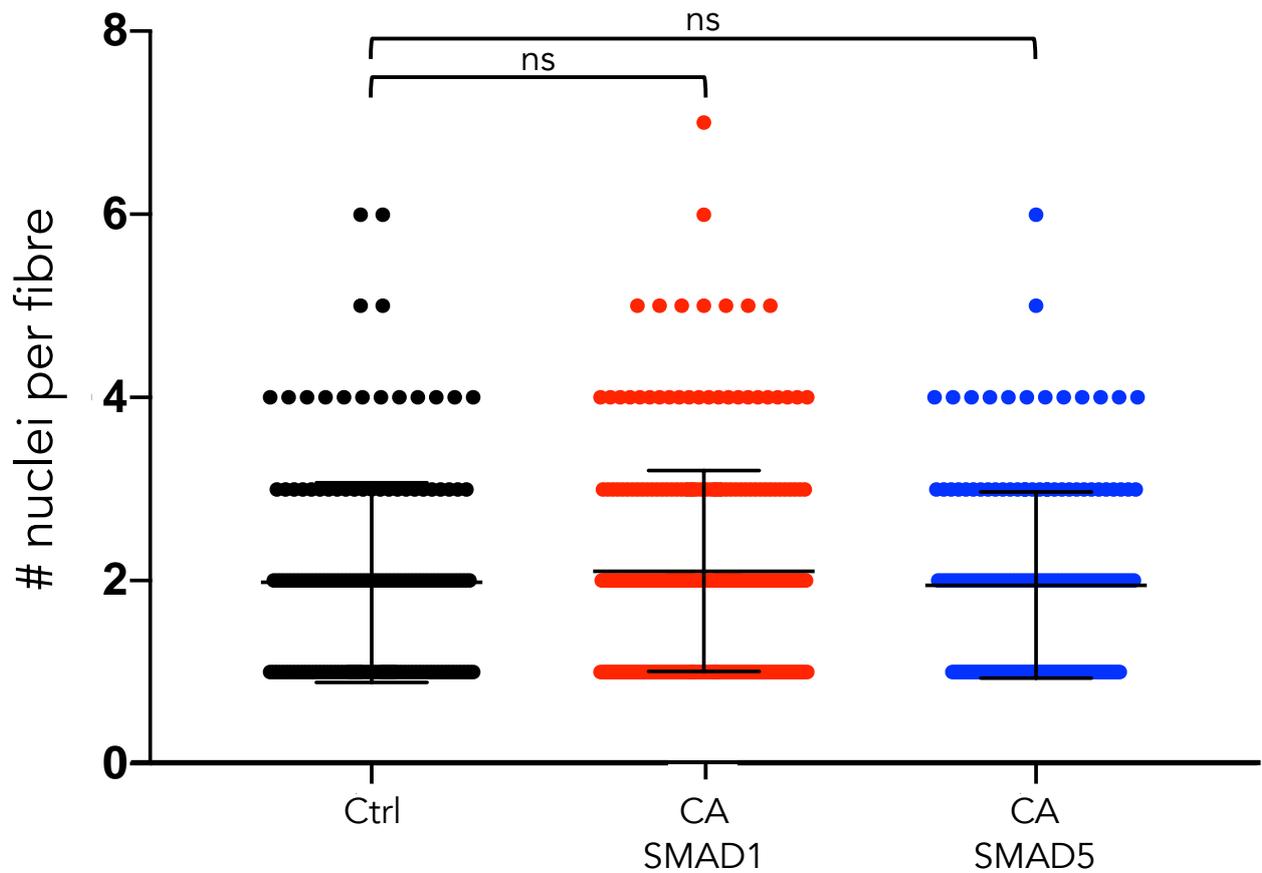
Supplementary Fig. 5

Myosin Light Chain promoter is expressed in terminally differentiated myocytes. **a**, Overlay confocal stacks of a single somite electroporated at HH15 (E2.5), and imaged at HH25 (E4.5). The white solid line delineates the somite. The dotted lines delineate the transition zone (TZ). **b**, Electroporation control, a CAGGS ubiquitous promoter driving nuclear H2B-RFP. Expression is observed in the dorsomedial lip (DML), the TZ and the myotome. **c**, Expression of GFP driven by a myosin light chain (MLC) promoter. No expression of the reporter is seen in the DML. Cells within the DML initiate MYF5 and MYOD expression²⁵ before entering the TZ. TZ cells are all postmitotic and express MyoG (our observation). Faint GFP expression was observed in a few cells of the TZ, while all myocytes of the myotome robustly expressed the fluorescent marker. MLC is therefore activated in terminally differentiating progenitors; **d**, Antibody staining against Myosin Heavy Chain (MyHC) showing expression exclusively in differentiated muscle fibres of the myotome. RFP and GFP were detected with IHC. Scale bars: 50 μ m.



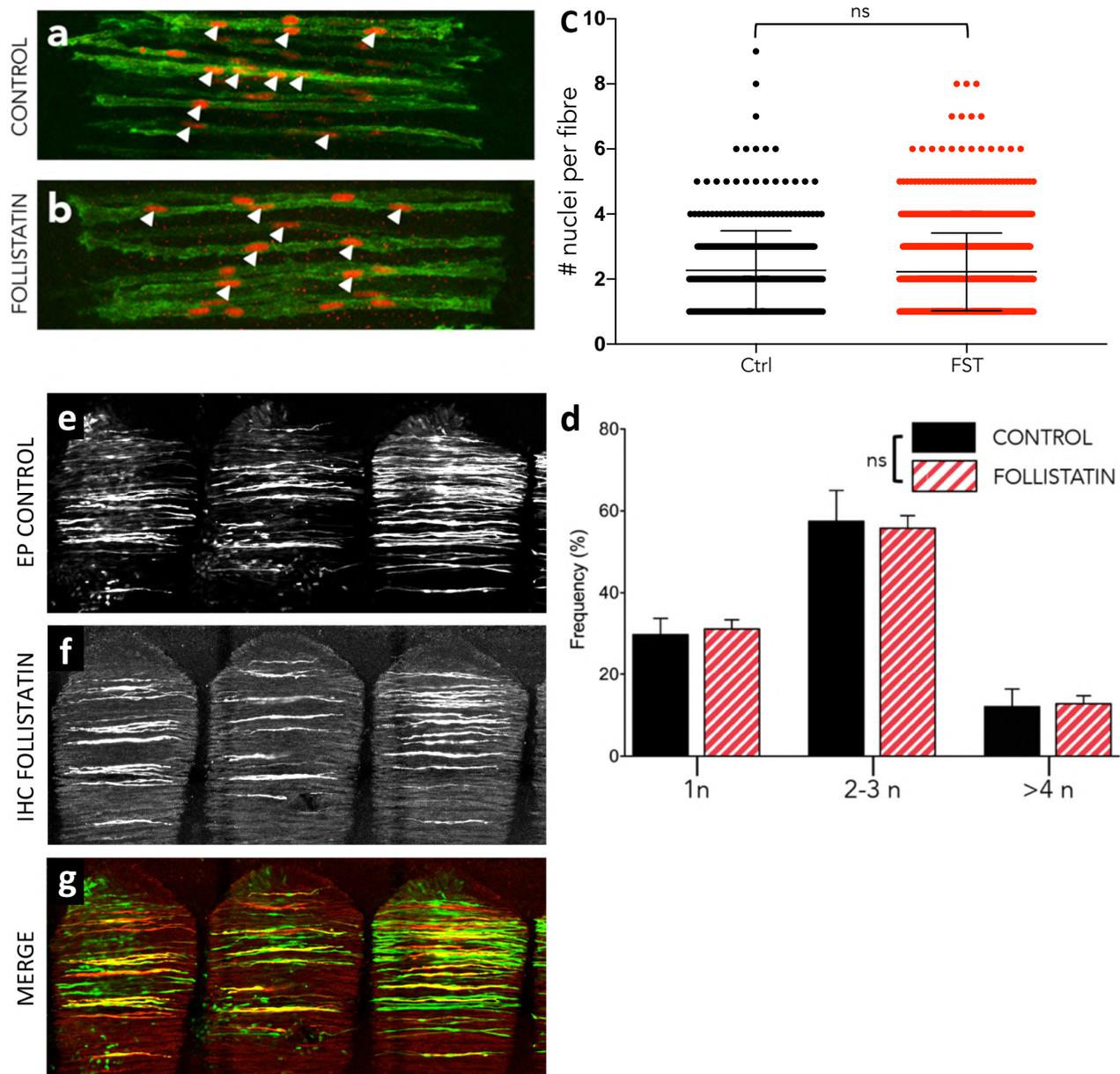
Supplementary Fig. 6

SMAD2/3-dependent TGF β signalling regulates myoblast fusion *in vivo*. Scatter plots showing the number of nuclei per fibre in each condition. Shown are means and standard deviations; ***P<0.001



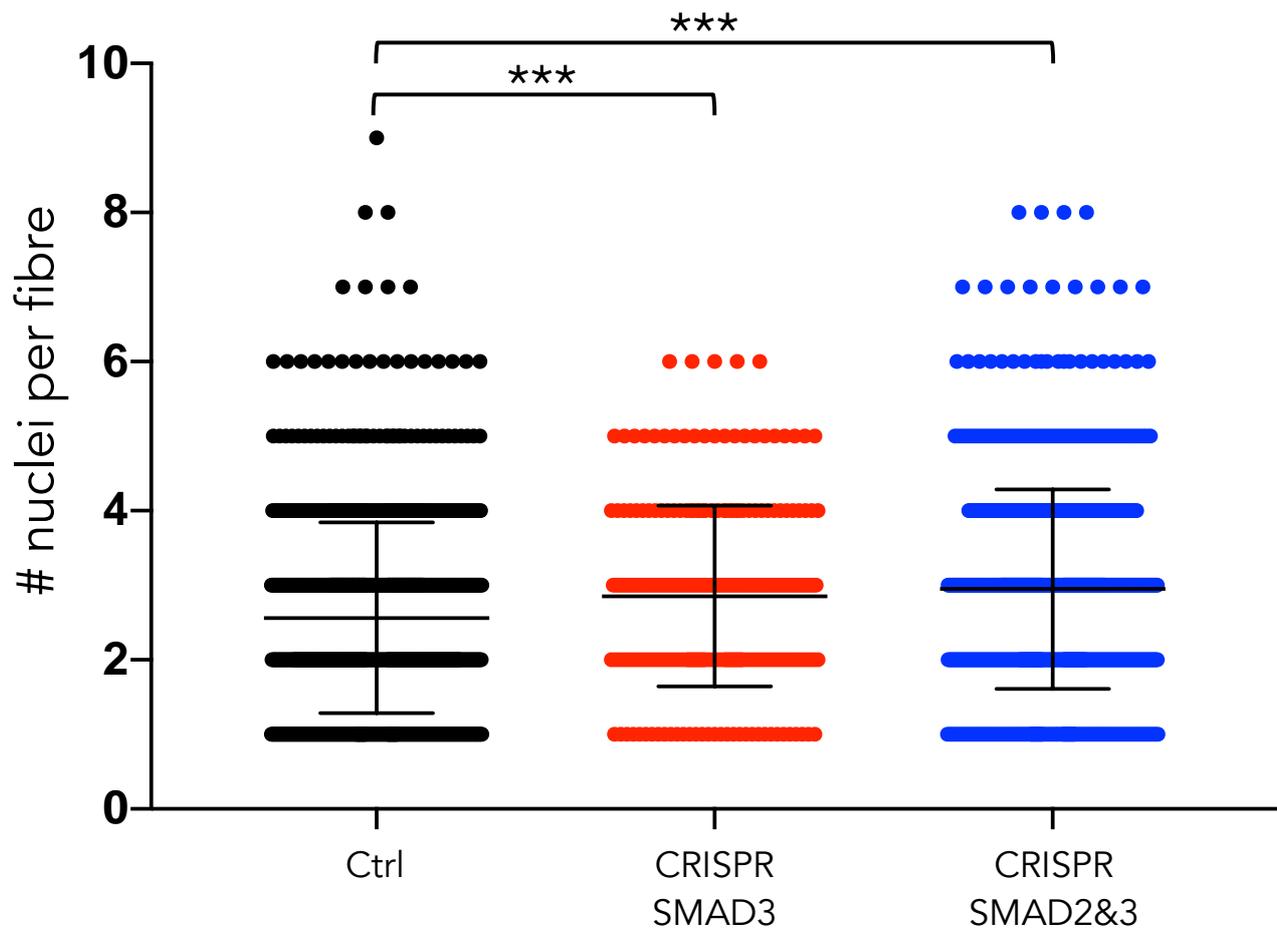
Supplementary Fig. 7

SMAD1/5/8-dependent TGF β signalling does not regulate myoblast fusion *in vivo*. Scatter plots showing the number of nuclei per fibre in each condition. Shown are means and standard deviations; ns: non statistically significant difference.



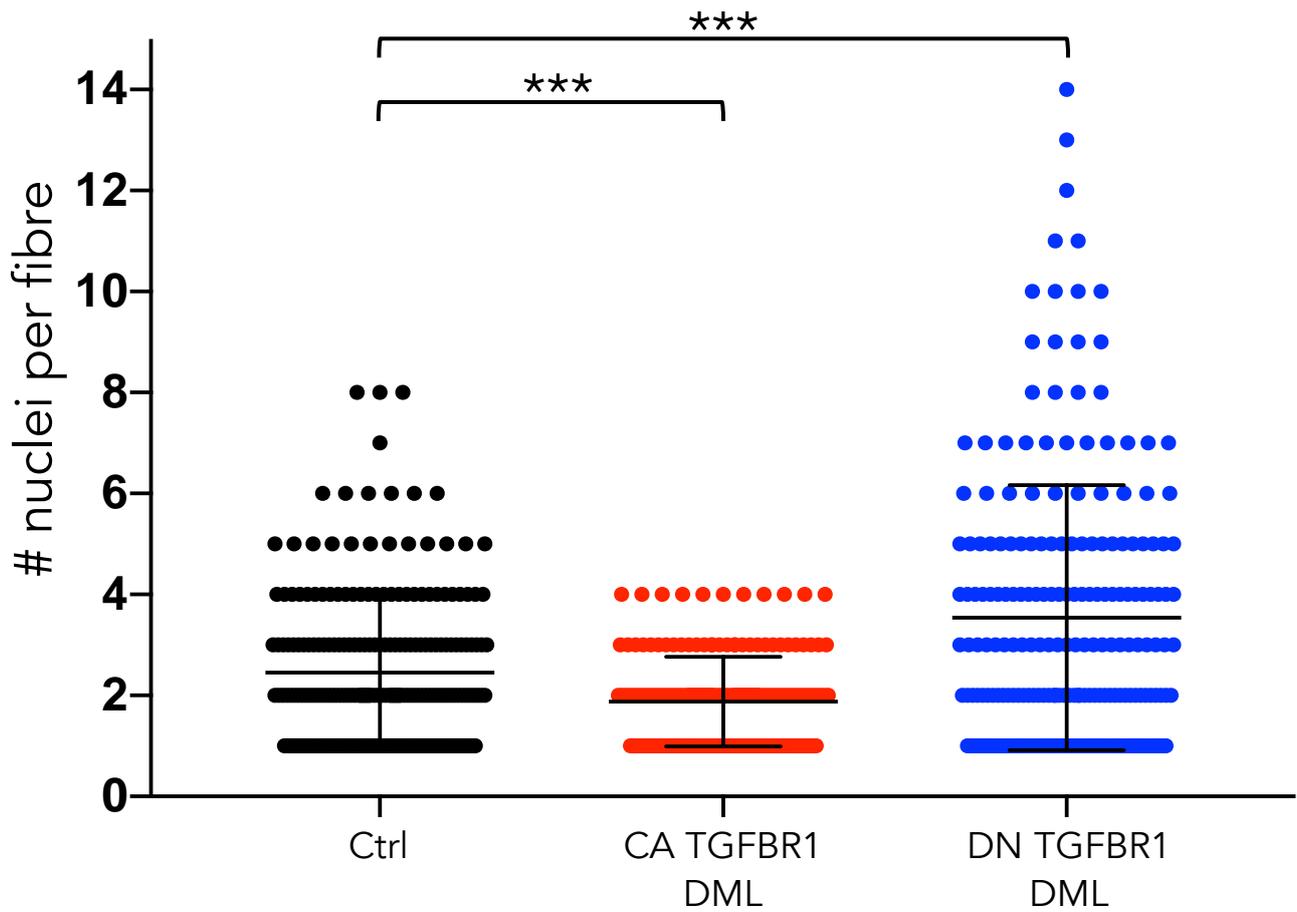
Supplementary Fig. 8

Myostatin does not regulate myoblast fusion *in vivo*. **a,b**, Dorsal views of confocal stacks of somites observed at E5.5 and electroporated in the DML at E2.5 with a plasmid coding for the Myostatin specific inhibitor Follistatin. Embryos were fixed and stained against GFP and RFP antibodies. **c**, Scatter plots showing the number of nuclei per fibre (means and standard deviations indicated). **d**, column graph for **a,b** showing the population of electroporated myocytes containing the indicated number of nuclei relative to their controls (in %). Error bars: SEM. White arrowheads point nuclei within fibres. **e-g**, Dorsal views of confocal stacks of somites observed at E4.5 and co-electroporated with a plasmid coding for BFP (as electroporation control) and for Follistatin and immunostained for FST (**e**). in **g**, merge. Statistical analyses: FST: \bar{x} : 2.23; n=53; Ctrl: \bar{x} : 2.27; n=20; P-value 0.46; Source data are provided (see Data Availability).



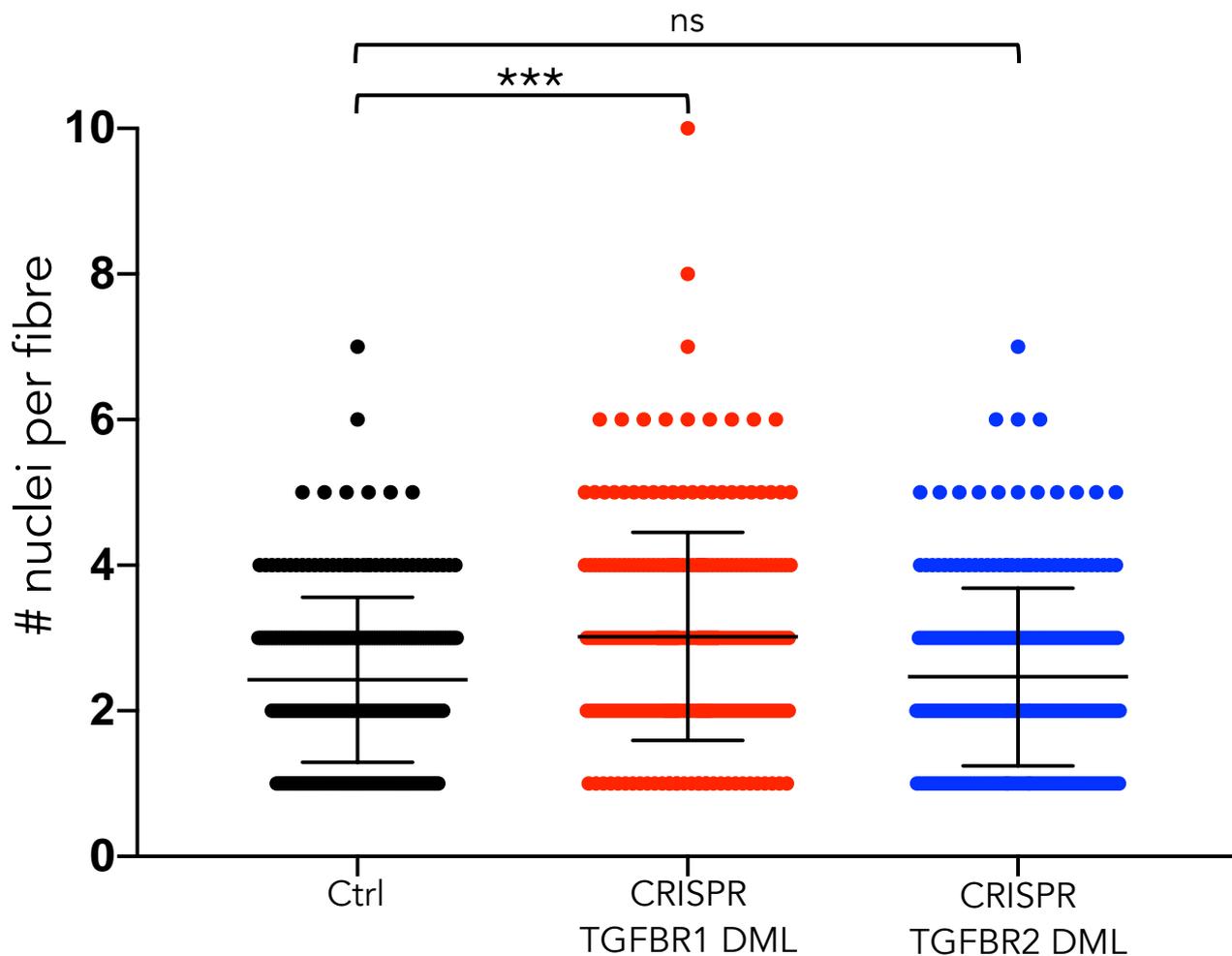
Supplementary Fig. 9

CRISPR-mediated loss of SMAD2 & 3 functions. Scatter plots showing the number of nuclei per fibre in each condition. Shown are means and standard deviations; ***P<0.001



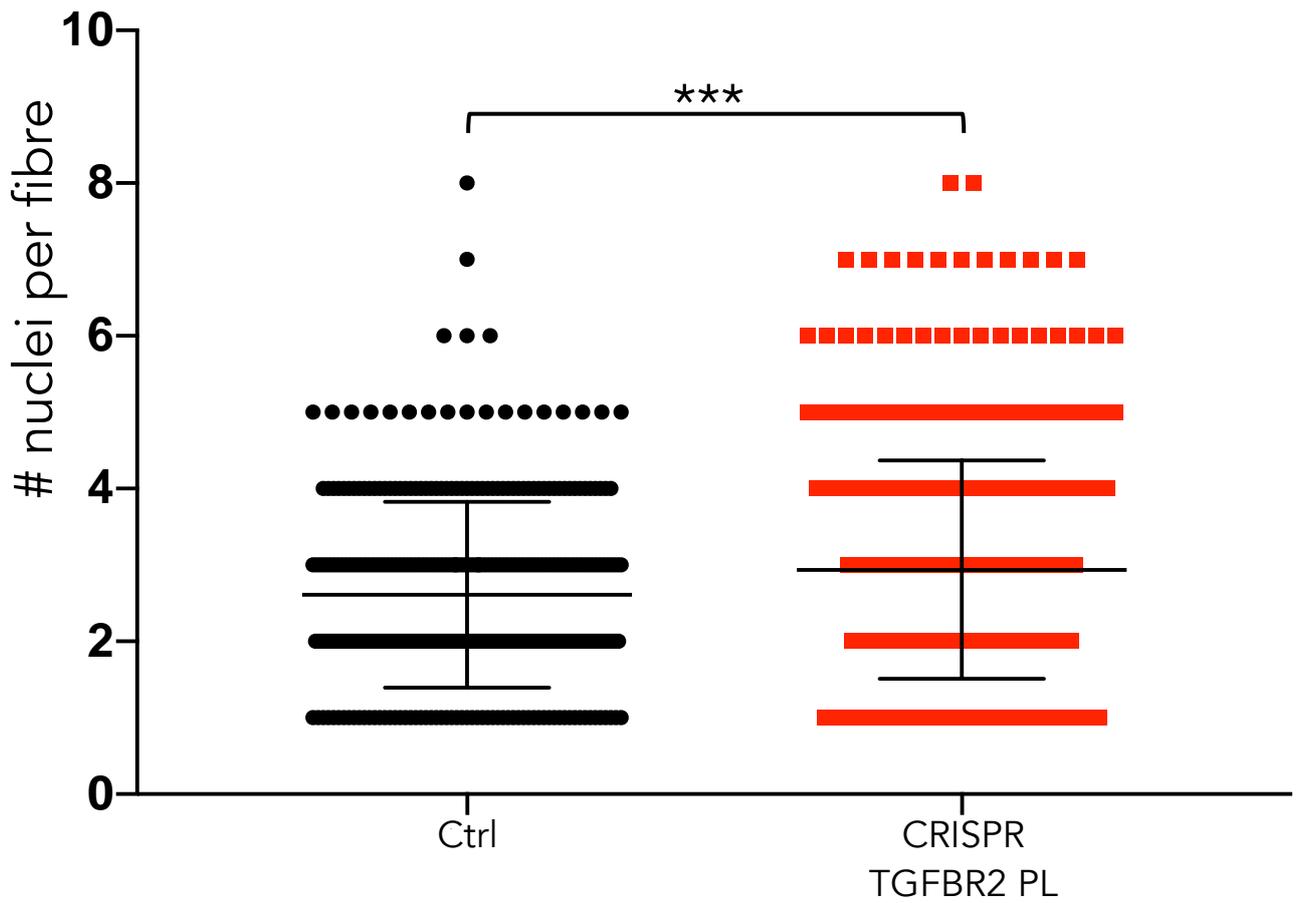
Supplementary Fig. 10

TGFBFR1 in DML-derived myocytes regulates fusion. Scatter plots showing the number of nuclei per fibre in each condition. Shown are means and standard deviations; ***P<0.001



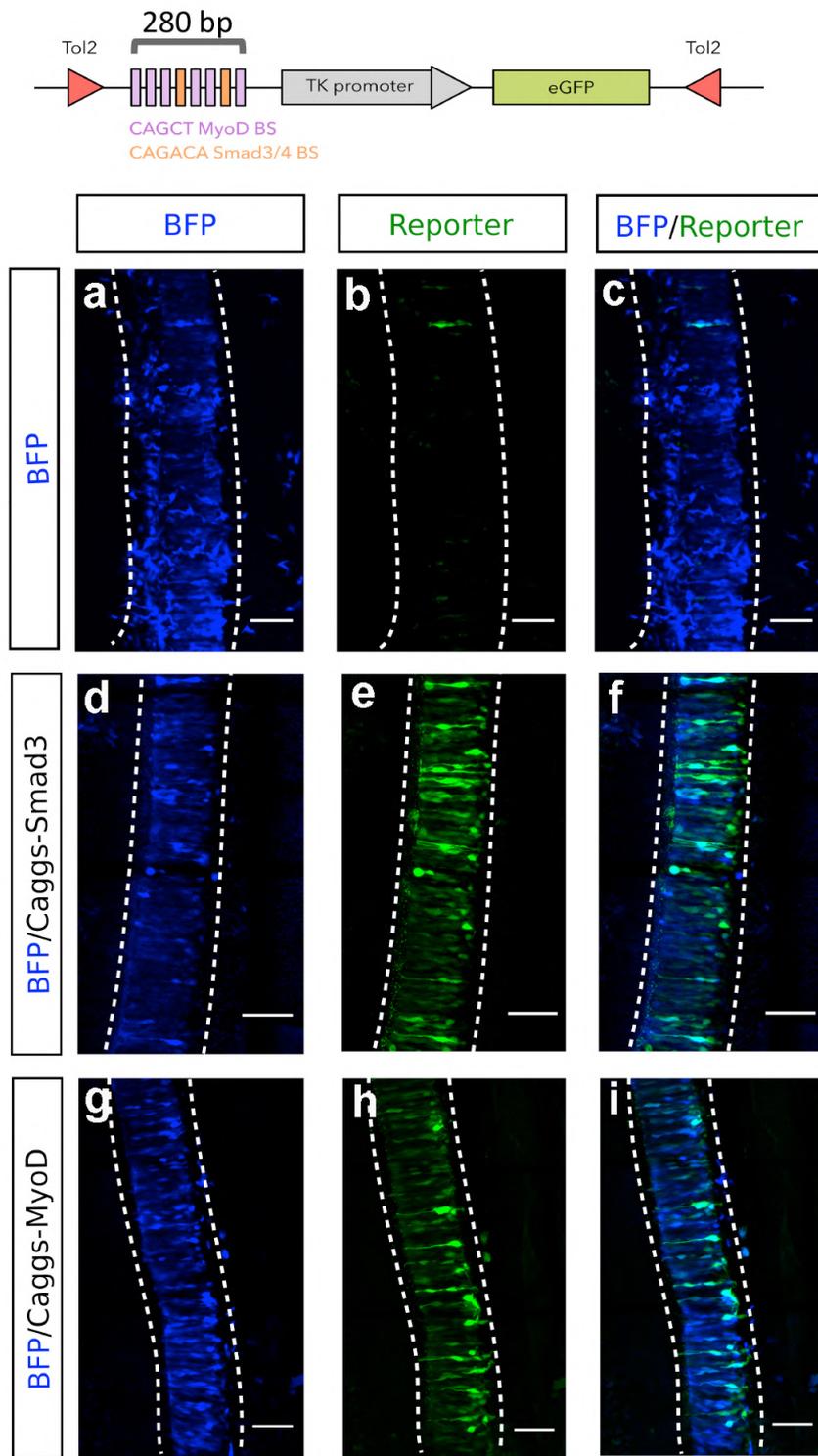
Supplementary Fig. 11

In the DML, TGFBR1 but not TGFBR2 regulates fusion. Scatter plots showing the number of nuclei per fibre in each condition. Shown are means and standard deviations; *** $P < 0.001$; ns: non statistically significant difference.



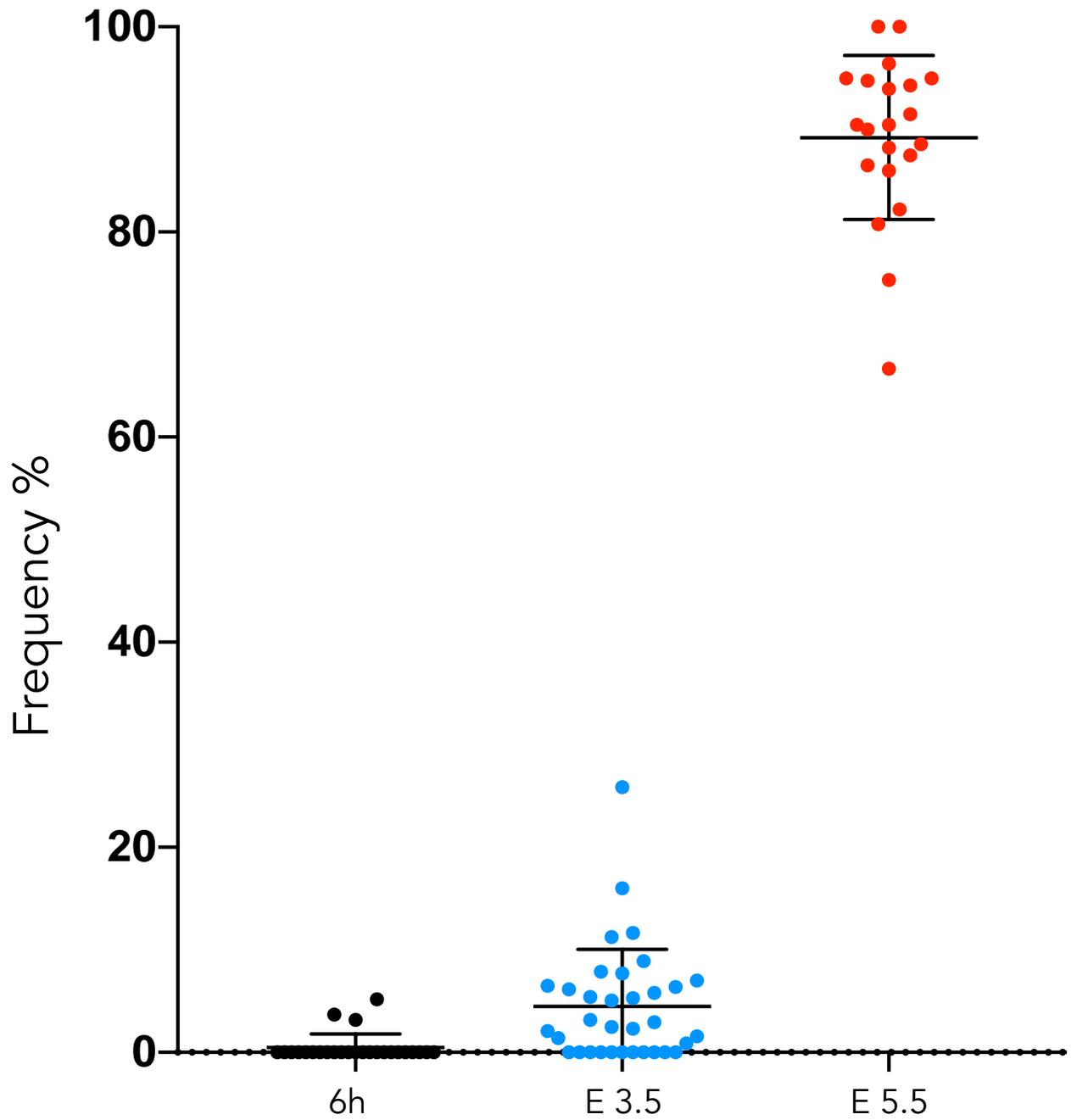
Supplementary Fig. 12

TGFBR2 in the posterior dermomyotome (PL) progenitors is necessary for fusion. Scatter plots showing the number of nuclei per fibre in each condition. Shown are means and standard deviations; ***P<0.001.



Supplementary Fig. 13

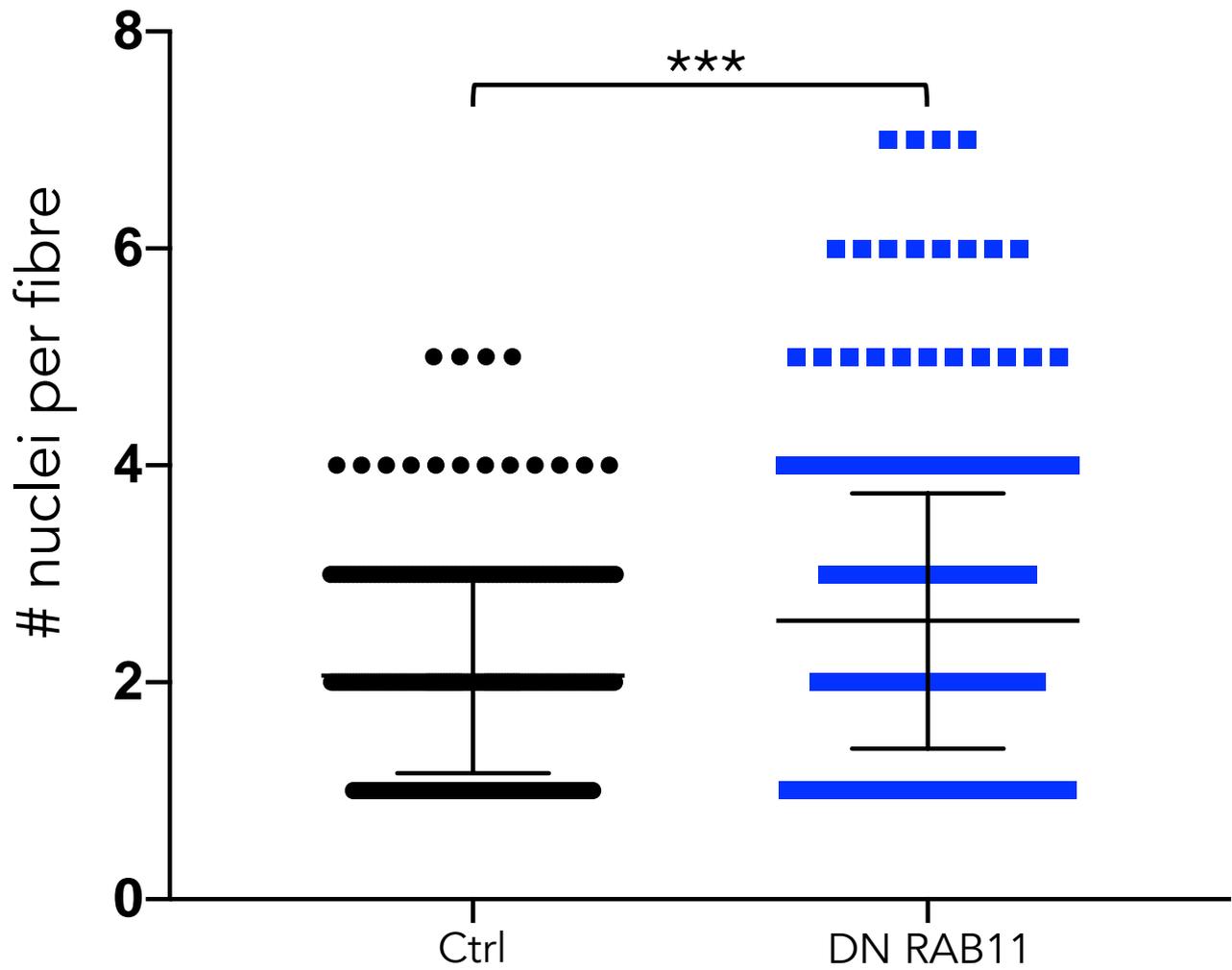
A new reporter to monitor SMAD3 activity in muscles. A 280 bp enhancer sequence located on mouse chromosome 11 between location 59161446 and 59161726, containing 6 MYOD and 2 SMAD3/4 binding sites, was cloned upstream of the TK minimal promoter and eGFP. **a-i**, Dorsal views of confocal stacks of one half of neural tube (delineated by dotted lines) co-electroporated with a plasmid coding for a CAGGS cytoplasmic BFP (in blue) and the reporter construct (in green). **d-f**, a CAGGS SMAD3 expression vector was added to the plasmid mix, resulting in a strong increase of the reporter activity. **g-i**, a CAGGS MYOD expression vector was added to the plasmid mix, also resulting in a strong increase of the reporter activity.



Supplementary Fig. 14

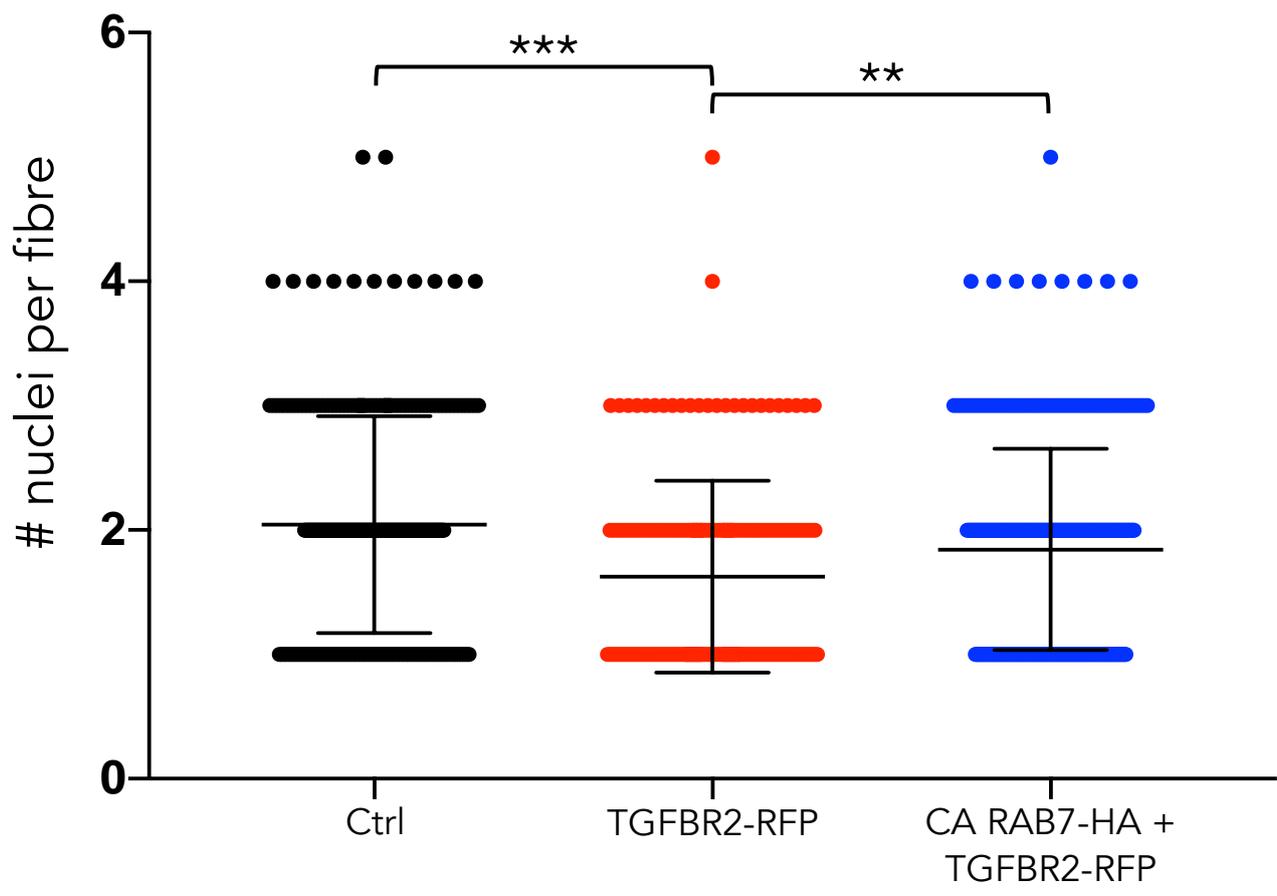
TGF signalling is activated upon fusion of PL-derived progenitors to DML-derived myofibres.

Scatter plots showing the proportion of electroporated cells activating the TGF β reporter 6 hours, one day or 3 days after electroporation. Shown are means and standard deviations.



Supplementary Fig. 15

Myoblast fusion is dependent upon RAB11 function. Scatter plots showing the number of nuclei per fibre in each condition. Shown are means and standard deviations; ***P<0.001.



Supplementary Fig. 17

Rescue of TGFBR2-mediated inhibition of fusion by CA RAB7. Scatter plots showing the number of nuclei per fibre in each condition. Shown are means and standard deviations; ***P<0.001.

Table 1. Genes inhibitors of C2C12 fusion

Entrez Gene ID	pvalue	%nuclei/myotube	pvalue	%nuclei	pvalue	%myogenin
Smad4	8,42E-15	525,54	0,14	98,56	2,20E-16	156,57
Fzd4	2,20E-16	508,18	0,00	111,16	2,20E-16	139,43
Mdfi	2,20E-16	432,86	7,11E-15	148,72	6,41E-10	114,60
Tgfb2	6,13E-13	405,95	0,00	111,81	2,20E-16	154,19
Mmp17	2,52E-13	400,40	0,00	115,77	2,20E-16	145,97
Adh5	2,20E-16	387,35	1,21E-09	126,46	2,20E-16	125,79
Sgcd	3,77E-11	386,64	4,84E-07	119,80	0,01	106,67
Cyfp2	1,53E-05	382,66	0,00	92,34	0,00	104,30
2310002L13Rik	1,20E-15	379,80	2,79E-12	129,66	2,90E-09	115,36
Smad3	2,20E-16	377,95	0,73	100,32	2,20E-16	136,95
Habp4	1,84E-14	375,01	2,05E-07	85,37	2,20E-16	152,15
Mrpl55	1,66E-12	372,03	0,00	123,44	1,05E-13	132,64
Pard6b	2,20E-16	360,21	2,20E-16	145,19	0,08	104,93
Inpp4a	4,51E-09	348,96	1,96E-07	121,85	3,89E-13	130,06
D17Wsu104e	7,58E-13	348,92	2,80E-06	112,08	2,20E-16	138,39
Ube2o	9,79E-13	340,50	0,22	104,49	6,23E-08	83,31
Srp54a	8,88E-12	334,81	2,57E-12	119,38	1,74E-06	111,15
Spaca1	1,74E-14	329,89	5,73E-12	131,26	6,11E-16	136,73
Best1	2,20E-16	324,30	6,24E-05	110,93	0,08	93,83
Rnf111	8,86E-16	319,44	0,25	103,26	2,20E-16	136,57
3110062M04Rik	2,85E-14	307,52	1,87E-05	113,99	2,72E-16	130,69
Dnaic1	2,96E-15	304,79	1,16E-09	121,40	2,20E-16	135,97
Osbpl8	2,20E-16	300,28	6,92E-06	115,59	2,20E-16	143,16
Qpctl	1,96E-11	295,90	8,18E-09	127,91	3,18E-15	128,44
Capn7	2,20E-16	295,42	6,03E-09	116,37	2,21E-14	122,16
6330416G13Rik	7,27E-16	293,11	4,84E-10	117,67	2,80E-05	109,34
Tgfbr1	2,06E-13	284,01	0,56	97,57	2,20E-16	148,79
Rrs1	9,95E-12	280,82	4,41E-07	117,55	2,20E-16	145,62
Fis1	4,01E-12	280,12	1,90E-11	132,70	1,31E-13	72,58
Csrnp1	7,00E-13	276,67	3,40E-14	134,42	1,85E-11	121,61
Ep400	2,55E-13	276,09	0,71	99,15	4,31E-14	125,76
Mpv17l	1,33E-14	275,41	0,00	110,83	2,20E-16	148,62
2410018M08Rik	4,33E-15	274,92	0,01	107,24	2,20E-16	130,66
Pcgf2	6,21E-13	274,88	3,39E-11	119,28	2,20E-16	139,64
Tnfsf13b	1,76E-09	271,19	0,33	102,68	3,62E-16	125,97
Fam167b	2,31E-11	271,15	5,74E-13	130,48	7,14E-16	126,39
Scamp2	1,51E-13	269,26	3,09E-10	124,17	0,01	107,04
Wipi2	1,60E-09	268,12	0,00	115,89	2,20E-16	136,86
Slc9a4	1,30E-14	266,91	0,61	101,61	2,37E-11	118,18
Gja10	7,42E-15	266,80	1,15E-09	118,39	2,20E-16	130,90
Tgds	3,08E-15	266,63	6,14E-06	113,59	2,20E-16	151,78
Mixl1	3,32E-14	266,55	6,42E-10	120,73	2,20E-16	131,39
Ahsa1	3,65E-11	265,95	0,12	102,81	2,20E-16	138,02
Apc2	4,38E-15	263,90	1,64E-10	120,29	4,82E-16	126,99
Abca9	1,03E-12	262,27	6,74E-07	116,35	1,43E-15	127,14
Pcmtd2	6,76E-12	261,94	0,03	108,37	2,20E-16	133,32
Rab35	1,15E-13	258,54	2,20E-13	123,68	2,20E-16	141,86
Fam122b	4,42E-16	258,27	3,00E-04	109,98	2,20E-16	129,91
Smarcb1	3,34E-11	254,57	0,83	101,59	2,20E-16	140,38
Acvr1b	2,02E-15	254,50	8,15E-06	111,56	2,20E-16	124,43
Rundc3a	3,62E-12	253,83	6,91E-11	118,88	2,99E-11	116,02
Plekha3	2,20E-16	253,54	3,03E-06	113,11	4,13E-11	115,93
Necap1	2,00E-15	253,42	4,26E-13	122,07	2,20E-16	136,07
Olf1189	1,17E-13	253,01	3,11E-06	114,19	2,20E-16	123,50
Ctps2	2,93E-06	252,82	0,00	115,25	2,20E-16	141,78
Lrit2	3,60E-16	252,38	0,00	112,53	3,84E-11	118,01
Gzf1	3,27E-13	252,11	7,47E-07	115,06	2,77E-15	124,70
Kcnj6	4,26E-12	249,45	1,85E-14	129,84	0,00	92,23
Asb13	6,09E-14	248,88	7,33E-07	113,51	8,29E-15	127,14
Nr1h5	1,94E-15	248,85	0,00	108,47	2,20E-16	135,59
Pcdhb7	3,28E-13	247,72	0,00	109,43	6,00E-16	129,21
Mcm6	3,02E-16	247,23	3,50E-09	118,13	2,20E-16	137,33
Alg5	2,02E-14	245,67	0,04	103,72	2,20E-16	138,85
Mut	7,92E-14	245,64	0,02	106,53	2,20E-16	141,59
Slc25a12	2,47E-13	245,47	4,34E-05	109,73	1,00E-15	125,34

Cbl11	6,92E-14	241,74	7,38E-05	111,77	2,43E-11	117,37
Ube4b	2,50E-12	240,76	0,36	97,13	0,13	103,00
Gdap2	1,92E-12	240,66	0,00	113,15	1,48E-12	121,31
Fgf1	8,63E-15	239,61	4,93E-16	128,92	2,20E-16	127,31
Rpap1	7,69E-15	238,91	7,64E-10	120,42	5,60E-08	112,73
Cul1	8,12E-11	236,19	0,02	105,28	2,14E-14	121,06
Ckap2l	1,42E-11	234,60	0,00	109,48	2,20E-16	142,69
Mrpl22	9,05E-15	234,45	4,00E-06	112,30	0,16	103,22
Bola3	1,60E-06	234,02	0,00	109,75	2,20E-16	138,64
Rufy3	1,11E-09	233,93	0,02	93,57	7,37E-16	123,18
Gm5089	1,75E-12	233,92	0,14	104,54	5,44E-15	127,90
Tmem47	1,54E-12	233,42	2,49E-07	129,11	3,56E-16	126,88
Ttll12	7,06E-07	232,20	0,01	108,55	2,20E-16	145,75
Caml	1,51E-11	231,77	0,84	99,07	1,13E-14	123,27
Vmn1r43	1,05E-10	230,54	7,30E-08	119,28	2,20E-16	130,60
Aldh16a1	8,22E-15	230,30	3,16E-13	124,11	0,00	107,76
Gjb5	1,37E-06	228,50	0,02	109,01	1,54E-14	134,06
Mum1	4,54E-14	228,00	0,00	109,40	3,20E-16	126,19
Fgf2	3,56E-10	227,57	0,09	102,42	4,81E-13	123,45
Dap3	1,14E-11	227,05	7,17E-06	114,62	0,00	107,35
Cdkal1	7,41E-12	226,30	1,98E-06	88,53	3,30E-08	114,47
Akap10	1,10E-11	226,09	3,08E-07	114,13	2,20E-16	133,61
Dgka	8,92E-14	225,20	0,04	106,02	2,14E-13	121,90
Masp2	3,53E-09	225,18	3,32E-05	111,50	6,02E-07	111,19
Mmgt1	1,18E-10	224,90	1,62E-12	124,16	1,01E-05	110,24
Irf7	4,23E-06	224,54	0,41	104,58	0,00	106,65
Lins	8,51E-10	223,44	0,00	109,12	2,20E-16	132,09
Pdcd6	1,53E-13	223,34	0,10	105,46	1,35E-12	123,57
4933425O20Rik	1,74E-14	221,19	2,09E-15	129,67	2,25E-08	113,93
Cd248	3,97E-12	220,04	3,18E-10	120,37	2,03E-15	127,05
Scube3	6,66E-06	219,78	0,00	108,60	3,66E-14	132,37
Clec4e	1,33E-08	219,18	0,21	103,71	2,20E-16	133,57
Tnfrsf26	8,83E-14	217,91	0,02	106,21	2,20E-16	142,27
Wdr8	9,40E-15	216,91	5,14E-10	117,41	1,01E-15	129,06
Kiss1r	1,33E-13	216,76	0,39	97,76	5,82E-13	119,02
Nudcd2	6,28E-10	216,74	0,05	106,28	0,05	104,80
Gpr135	1,06E-09	216,54	9,46E-05	109,50	5,59E-15	123,38
Tdrd7	2,64E-07	216,27	0,78	99,20	7,64E-15	129,02
Chic1	2,85E-13	215,72	0,00	115,87	2,58E-15	126,13
Tlr8	5,83E-11	215,20	0,00	108,48	1,80E-13	121,98
D19Wsu162e	5,41E-14	214,93	2,87E-10	118,31	1,52E-11	118,83
Bdh2	8,92E-10	212,16	0,00	106,84	7,18E-11	116,74
Hs3st1	1,73E-13	212,02	1,81E-09	119,15	2,24E-13	121,89
Ptgr1	6,29E-12	211,36	3,77E-05	111,21	5,02E-13	121,05
Arx	1,57E-11	210,95	9,47E-05	110,99	6,37E-13	122,32
Cc2d2a	7,03E-14	209,96	1,38E-05	112,50	2,20E-16	130,71
Pcgf3	1,34E-09	209,77	0,62	98,79	1,76E-14	125,62
Chst14	9,16E-09	209,44	0,67	100,04	6,50E-14	123,98
Zfp101	3,49E-13	207,42	0,02	106,45	2,20E-16	131,29
Itgb4	4,30E-11	207,41	0,04	105,38	3,82E-14	121,99
Tmem126a	5,76E-14	206,82	1,30E-06	111,71	2,20E-16	128,10
Maged1	1,21E-12	206,30	0,02	104,59	2,64E-14	120,52
Il1rl2	1,50E-09	206,09	0,57	101,63	1,42E-13	124,01
Ccdc50	3,20E-12	205,41	0,77	101,20	3,14E-15	124,27
Srp9	7,02E-13	203,99	0,12	103,84	2,20E-16	125,95
2510006D16Rik	2,65E-10	203,06	0,23	96,92	1,66E-14	125,41
Plp2	9,83E-12	202,56	1,68E-06	114,97	2,39E-11	119,54
Mael	1,86E-09	202,32	6,35E-05	113,28	1,12E-11	118,24
Chuk	3,35E-10	201,89	0,00	107,80	0,66	100,27
Gapvd1	8,21E-10	201,79	5,18E-06	112,49	4,79E-11	122,60
Mon1a	1,53E-08	201,21	2,56E-09	116,74	0,96	100,43
Fbxo9	1,33E-10	200,50	0,00	109,60	5,06E-06	111,77
Fam136a	2,09E-10	198,72	0,00	110,83	4,29E-07	111,56
Tgfb3	1,17E-10	198,32	0,19	97,46	3,26E-10	117,66
Mmadhc	2,77E-12	198,30	0,02	104,83	2,20E-16	126,17
Nfkbia	9,99E-14	197,96	0,58	100,75	7,18E-15	126,20
Nudt3	1,34E-11	197,40	0,02	105,79	2,20E-16	127,21

Eps15	1,40E-10	196,67	1,09E-06	112,99	4,51E-11	116,98
Ttll9	2,28E-05	194,84	5,88E-10	119,65	0,70	100,69
Stk11	2,58E-09	193,34	0,02	106,17	8,00E-13	121,24
Bbs10	1,89E-08	193,07	6,67E-07	114,47	0,00	92,19
Cnnm4	7,07E-12	192,83	0,15	103,62	1,19E-14	124,09
Pskh1	1,64E-07	192,58	0,17	97,27	8,27E-07	111,37
Dclre1b	6,98E-10	191,54	0,00	91,71	4,17E-16	123,05
4930590J08Rik	1,89E-08	191,52	2,32E-05	90,00	5,51E-05	110,82
Blnk	0,00	191,29	0,00	94,01	5,24E-06	89,09
Mzt1	2,41E-08	190,96	0,07	95,79	6,05E-14	122,68
Slc38a3	3,24E-06	190,90	0,00	108,84	0,01	93,27
Clpb	1,15E-08	190,06	3,25E-06	114,10	1,38E-08	114,71
Myocd	9,99E-12	189,54	0,01	108,59	4,55E-09	116,03
Pram1	7,29E-09	189,41	0,00	110,61	8,20E-13	120,51
Snx16	2,51E-05	188,92	0,90	100,14	2,96E-16	130,89
Cdkn1a	6,44E-09	188,46	4,22E-12	122,56	2,20E-16	62,96
Cfh	1,13E-08	188,34	1,19E-06	89,40	1,91E-10	115,91
Cpeb4	1,10E-10	188,27	0,08	105,39	1,53E-11	116,17
Pak1ip1	1,75E-12	187,84	9,84E-10	116,90	0,00	107,83
Nfatc2	1,47E-11	186,19	2,46E-05	122,86	7,75E-09	132,65
Unc45a	1,68E-10	186,01	0,00	108,44	1,17E-12	79,20
Arhgap23	5,91E-11	185,84	0,04	103,90	1,31E-12	119,98
Rhbdl3	2,29E-09	182,95	0,50	98,85	9,06E-13	127,75
Tgfbr3	1,30E-09	182,53	0,00	106,43	1,26E-12	120,42
Mas1	4,80E-09	182,39	0,45	98,47	2,20E-16	124,31
Camkk2	2,64E-07	181,83	0,08	105,43	0,17	102,34
Hmgcl1	2,00E-07	181,52	0,40	99,65	1,69E-13	125,16
Fabp3	1,01E-09	179,79	1,34E-06	112,38	4,04E-05	109,44
Acp2	1,27E-08	179,65	0,42	102,51	9,37E-10	116,83
Tmem128	4,92E-09	179,07	0,01	93,77	1,05E-07	112,22
Nt5c	2,27E-05	178,19	0,06	95,51	2,09E-10	118,00
Rnf130	2,55E-08	177,38	1,69E-07	88,06	3,68E-11	116,82
Smg6	6,28E-09	177,29	2,63E-15	76,95	0,04	124,29
Aqp9	7,25E-08	177,00	0,73	100,57	7,78E-10	115,65
Cr1l	1,33E-07	174,85	0,00	91,70	0,08	103,29
Rapgef4	1,07E-06	173,99	0,79	98,65	2,34E-10	117,74
Btbd17	9,27E-07	173,71	0,14	103,95	1,03E-07	111,74
Fads1	3,59E-09	173,25	0,53	98,49	0,00	107,46
Idh3a	6,47E-08	173,16	0,98	100,91	2,41E-10	117,84
Muted	3,02E-07	172,42	0,19	96,72	0,08	94,51
Gsto1	8,41E-08	172,27	0,02	107,12	7,54E-07	111,63
Siglece	8,20E-05	172,16	0,13	95,97	5,70E-12	119,72
Atg14	3,45E-06	171,65	0,00	108,54	0,01	106,93
Mknk2	5,19E-05	170,96	1,14E-08	85,80	2,63E-15	124,61
2610301B20Rik	8,37E-07	169,43	0,77	100,59	7,77E-08	111,88
Man2b1	6,93E-09	168,06	9,77E-05	91,26	2,82E-08	113,47
Ptcd1	1,85E-08	167,17	0,51	102,30	0,00	107,68
Fbxl3	8,38E-09	166,61	0,01	107,39	0,25	103,23
Dmrt2	5,63E-08	166,15	0,41	101,54	6,42E-05	90,60
Tmem35	2,22E-08	164,93	0,08	95,77	0,00	107,30
Tex19.1	4,58E-08	164,67	6,23E-08	112,55	1,21E-08	86,72
Arl4c	2,42E-05	164,31	0,09	104,42	1,91E-06	110,43
Map3k2	2,36E-05	164,01	0,80	101,31	0,27	102,75
Iffo2	2,72E-05	163,50	0,04	94,87	0,08	104,63
Drg2	1,83E-07	161,47	0,12	103,72	0,84	99,54
Ak4	2,46E-05	160,36	0,06	105,65	3,24E-11	81,63
Reep1	1,26E-08	159,79	0,06	95,21	0,09	95,82
Mapkapk2	5,41E-06	159,12	0,65	101,06	0,00	92,54
Fbxo18	1,31E-05	157,98	0,24	103,29	0,79	100,80
Atg4c	1,75E-07	157,51	0,15	96,09	0,00	90,41
Olf986	4,57E-08	157,03	0,00	91,86	0,01	105,07
Calcr1	0,00	154,85	0,02	106,39	0,08	95,27
Tgm2	7,88E-05	154,75	1,15E-07	85,95	9,49E-14	124,90
2610528E23Rik	1,38E-08	153,73	0,00	108,57	8,09E-06	89,84
2700049A03Rik	9,00E-07	152,25	2,45E-12	82,28	3,79E-10	114,43
Zfyve1	1,04E-06	151,14	2,28E-05	111,79	0,02	94,37
Efhc1	9,54E-06	147,46	6,28E-07	88,09	0,88	99,69

Tas2r126	0,00	145,69	1,30E-07	86,36	4,52E-09	85,10
Mthfd2	0,00	145,20	0,15	103,75	0,18	94,83
Myo1e	1,91E-05	141,26	0,77	100,14	0,17	96,14

Supplementary Table 1

Scores (as normalized to 100 to the effect of negative controls, RLuc and FLuc), accompanied with their respective P-values, obtained after esiRNA transfection of the indicated genes into the mouse myogenic cell line C2C12. Effects on fusion (nuclei per fibre, in red), proliferation/cell survival (nuclei count, in blue) and myogenic differentiation (myogenin expression, in green) after loss of function of those molecules are indicated. The list encompasses the gene names (Entrez gene IDs) of the esiRNAs that obtained the highest score in the fusion assay. The corresponding genes are therefore predicted to act as strongest inhibitors of C2C12 fusion.

Table 2. Genes necessary for C2C12

Entrez Gene ID	pvalue	%nuclei/myotube	fusion		%nuclei	pvalue	%myogenin
			pvalue	%nuclei			
Mrps10	2,20E-16	34,13	1,20E-15	77,32	3,89E-12	76,46	
Tfrc	2,20E-16	34,95	2,20E-16	63,29	2,20E-16	69,01	
Fau	2,20E-16	38,18	3,15E-16	77,16	7,78E-14	77,76	
Psmc6	2,20E-16	39,28	5,92E-16	74,56	2,20E-16	66,09	
Snrpd1	2,20E-16	40,39	2,20E-16	74,32	1,58E-11	79,81	
Eif2s1	2,20E-16	44,04	3,26E-11	82,37	0,01	92,34	
Prpf8	2,20E-16	44,35	2,24E-06	87,23	1,07E-09	85,17	
Sdad1	2,20E-16	44,99	2,20E-16	72,94	0,02	94,73	
Dcaf13	2,20E-16	45,03	2,48E-14	76,98	9,57E-10	84,08	
Mprrip	2,13E-15	45,33	5,95E-09	84,22	4,79E-13	77,27	
9830001H06Rik	2,20E-16	45,33	0,01	104,89	1,66E-06	90,10	
Klhdc3	2,20E-16	46,10	0,67	100,54	1,42E-15	76,26	
Hmx1	2,20E-16	46,15	8,88E-08	87,17	3,13E-14	80,67	
Sec24a	2,20E-16	46,51	4,22E-08	86,35	3,47E-13	81,26	
Atp7b	5,72E-16	46,91	0,01	93,72	3,38E-08	86,39	
Hook3	2,07E-15	47,42	1,44E-13	81,99	2,20E-16	72,09	
Mapk14	1,10E-14	47,61	2,63E-09	86,96	2,20E-16	71,97	
Edf1	1,06E-15	48,02	0,01	107,11	2,20E-16	57,52	
Olf1321	7,73E-15	48,18	7,56E-14	79,04	2,01E-06	87,60	
Ubap2l	2,20E-16	48,35	1,04E-11	83,36	6,74E-06	87,17	
Zfp513	2,04E-14	48,50	4,44E-12	80,91	3,15E-09	83,70	
Tmem40	1,85E-15	48,56	0,86	99,76	3,50E-16	70,35	
AA467197	8,38E-15	48,73	0,00	107,42	1,24E-11	82,15	
Ubxn10	1,48E-14	48,78	0,00	89,80	0,00	90,70	
Gnl3l	1,50E-15	48,95	7,69E-15	73,72	0,00	85,27	
Micalcl	2,25E-15	49,06	3,09E-10	82,43	0,00	108,67	
Plcg2	7,19E-16	49,09	8,74E-11	83,13	5,45E-13	82,29	
Rgmb	2,20E-16	49,23	0,45	101,30	0,46	99,16	
Katnb1	1,56E-15	49,23	4,16E-08	86,79	5,76E-06	89,17	
Prrc2c	2,18E-13	49,54	2,93E-15	75,56	0,22	96,23	
Gsk3b	1,94E-15	49,62	3,13E-06	88,71	6,85E-14	77,28	
Asap1	3,32E-14	50,45	1,12E-08	85,22	4,55E-15	77,80	
Dhx30	1,48E-14	50,61	2,30E-08	84,81	0,00	107,52	
Pknx1	4,13E-15	50,90	9,86E-14	78,18	0,00	90,17	
Blm	1,38E-13	50,91	1,37E-06	87,50	3,83E-12	79,99	
Bnip2	4,29E-14	51,39	5,46E-08	85,91	3,93E-05	89,26	
Exoc6b	4,75E-13	52,05	0,02	106,49	2,07E-06	89,12	
Plekhf1	1,83E-12	52,39	9,24E-09	84,48	4,64E-11	79,86	
Catsper2	4,96E-14	52,48	0,01	93,97	0,64	98,39	
Dnalcl	1,24E-14	52,79	1,88E-06	89,89	1,28E-07	88,26	
Lingo2	3,43E-13	53,13	4,18E-07	87,23	0,00	91,84	
C1ql3	1,33E-12	53,19	6,11E-10	83,84	0,00	88,93	
Eci2	4,98E-13	53,24	0,40	101,42	0,50	99,18	
Atp6ap1	5,08E-13	53,75	9,39E-10	84,93	2,39E-07	113,69	
Arhgap30	1,49E-13	53,79	0,77	98,65	3,90E-07	113,62	
Crls1	5,77E-14	54,44	2,82E-05	110,84	1,10E-07	114,62	
Brca2	2,60E-12	54,49	3,92E-11	83,04	0,00	92,61	
Melk	2,23E-14	54,69	0,04	95,16	8,23E-09	86,99	
Eci1	2,90E-14	55,44	0,30	97,41	1,06E-08	112,49	
Olf1040	1,82E-12	55,71	2,18E-09	85,89	3,00E-05	88,05	
Amz1	4,71E-12	55,96	0,07	95,25	2,23E-07	88,52	
Tmem87a	1,03E-12	56,51	2,23E-14	77,29	0,00	93,07	
Prkca	2,61E-13	56,62	1,22E-09	84,68	2,80E-09	86,59	
Atp6v0e	1,55E-11	56,92	4,11E-08	87,00	1,97E-06	89,53	
Sema5b	5,62E-12	57,00	3,22E-10	84,15	2,86E-10	84,13	
Srsf1	7,37E-12	57,25	1,72E-10	83,92	0,02	106,10	
Arhgap17	2,63E-13	57,38	0,00	92,92	0,01	105,00	
Jarid2	1,57E-13	57,40	0,37	97,24	8,98E-10	83,56	
Tmem186	4,23E-11	57,54	0,02	106,17	0,00	106,47	
Trim45	3,97E-12	57,64	2,97E-09	85,88	0,88	99,66	
Idua	3,27E-10	57,85	2,56E-11	80,31	3,41E-14	124,50	
1700001O22Rik	4,91E-09	58,19	0,97	100,94	0,00	88,76	
Gria4	5,67E-10	58,21	0,24	97,70	1,06E-14	75,38	

Cct8l1	3,11E-11	58,38	1,75E-08	84,59	5,27E-09	86,07
Triobp	2,37E-12	59,10	6,77E-07	89,98	1,66E-13	81,21
1110008J03Rik	1,14E-11	59,46	0,02	93,29	2,78E-13	78,42
Rinl	1,08E-11	59,63	0,00	91,35	8,60E-07	89,17
Specc1l	5,92E-11	59,87	0,11	104,26	0,93	99,75
Pkd1l2	4,30E-10	60,41	1,87E-08	84,77	0,41	101,97
Taf15	3,04E-11	61,06	0,00	91,21	0,17	97,18
Chmp4c	2,89E-09	61,11	0,07	105,50	1,00E-06	88,23
Gpr30	9,29E-11	61,23	0,11	96,80	6,05E-12	81,99
Zfp697	3,92E-10	61,33	7,61E-06	88,81	5,54E-06	86,81
Smpd4	1,02E-10	61,70	0,01	106,34	0,00	94,52
Creb3l4	2,83E-10	61,71	0,49	101,58	4,35E-12	80,25
Cep97	1,21E-10	62,00	1,14E-06	88,62	8,24E-06	89,03
Slc39a1	5,36E-10	62,04	0,09	95,26	0,17	97,23
Sall4	2,28E-09	62,05	0,29	95,87	6,24E-13	80,52
Ruvbl2	1,67E-09	62,20	0,46	98,00	6,21E-07	88,61
Haus8	2,53E-09	62,21	0,53	100,78	0,00	92,19
Lrmp	3,37E-09	62,21	0,05	95,52	9,58E-09	85,86
Dnajc24	2,39E-10	62,22	2,22E-09	85,50	0,01	94,89
Psrc1	3,95E-10	62,40	1,15E-06	89,58	0,61	101,64
Rgs5	2,05E-10	62,65	0,00	110,90	0,00	92,95
Nlgn2	4,48E-10	62,78	0,00	92,14	0,00	92,54
Hoxc5	9,63E-10	63,07	0,47	97,52	6,44E-12	82,57
Ezr	5,81E-09	63,19	0,02	94,03	2,45E-05	88,73
Fktn	3,35E-09	63,19	4,51E-06	87,41	0,66	100,94
Kif3c	1,58E-09	63,26	0,06	95,91	9,46E-11	84,46
Ncoa2	1,95E-09	63,33	0,00	92,57	2,22E-08	86,14
Wwtr1	1,39E-10	63,47	0,01	92,94	6,57E-14	120,73
Hmbs	2,53E-09	63,71	8,05E-14	127,11	2,58E-05	90,81
Zdhhc25	3,48E-09	63,74	0,00	106,43	1,27E-06	111,36
Fam125a	6,60E-10	63,77	9,67E-08	111,96	2,76E-12	81,19
Bag3	7,84E-10	63,85	0,00	107,11	6,55E-07	88,63
Itgae	2,18E-09	64,04	0,00	108,79	7,97E-07	88,10
Mepce	2,16E-08	64,05	0,10	103,55	2,49E-06	110,18
Irak1	8,26E-10	64,19	2,22E-10	118,00	1,55E-11	84,00
Tspan7	1,19E-09	64,24	0,54	98,36	0,13	97,09
Rcsd1	3,52E-09	64,31	0,31	97,48	4,71E-07	112,66
Dennd4b	1,82E-08	64,43	0,00	92,78	0,71	101,01
Idh1	7,06E-09	64,76	3,99E-05	89,52	0,01	104,90
Psmc5	2,51E-08	64,78	0,56	101,33	0,00	91,91
Zfp341	3,49E-09	64,79	0,00	91,49	0,01	94,48
Dnajb5	1,81E-08	65,18	0,35	97,96	0,01	94,72
Fgf3	8,80E-09	65,32	5,90E-09	120,01	0,01	105,52
Rab39	5,48E-08	65,33	0,39	101,87	0,01	93,02
Tcf15	3,37E-09	65,46	0,00	108,27	1,60E-06	87,42
Ppp2r1a	2,70E-08	65,56	0,08	95,76	3,12E-05	90,72
Cnot6l	9,02E-08	65,57	0,22	102,16	0,02	95,61
Gm2a	2,54E-08	65,61	0,58	98,74	0,00	91,82
Srrm2	2,30E-08	65,70	0,87	99,24	0,02	94,13
Tinf2	2,81E-08	65,89	0,02	94,45	0,23	102,17
Cldn13	1,31E-08	65,95	3,35E-10	121,81	4,20E-06	109,84
1810037I17Rik	3,54E-08	66,07	0,13	96,56	8,33E-09	85,14
Syt7	3,68E-09	66,16	0,00	105,22	0,00	92,93
Vta1	5,68E-08	66,18	0,09	96,15	0,00	92,64
Reep2	1,71E-07	66,27	0,00	91,91	0,01	94,36
Galntl6	1,87E-07	66,38	3,53E-12	80,75	0,01	105,62
Rhod	1,35E-07	66,42	0,00	93,24	0,00	108,82
Cpsf1	6,47E-08	66,54	1,26E-05	111,04	0,04	96,26
Zbtb46	1,65E-08	66,81	4,57E-09	86,19	0,56	100,76
Apba1	9,07E-08	66,86	0,02	95,05	0,37	103,25
Syt5	2,79E-08	66,95	0,04	95,06	0,03	94,98
Grpel1	1,45E-07	67,09	0,35	101,64	3,76E-06	89,89
Metap2	8,05E-07	67,12	0,03	92,58	0,09	95,98
Cenpb	6,87E-08	67,12	0,03	95,33	0,22	97,58
Bcor	4,40E-08	67,15	5,77E-07	88,41	0,31	98,01

Usp29	1,17E-07	67,68	0,09	96,18	0,00	108,15
Trem11	4,17E-07	67,82	0,26	102,26	0,56	98,89
Fxyd5	1,10E-07	68,03	4,03E-05	89,70	0,15	96,18
Kcnh3	7,05E-07	68,05	0,00	91,25	0,12	97,07
Aoc3	1,76E-07	68,06	0,00	109,96	8,57E-07	113,09
Fhl1	2,03E-06	68,09	0,00	110,02	2,01E-08	84,38
Ncapd3	3,25E-07	68,45	0,79	98,62	2,25E-07	113,36
Plat	2,39E-06	68,49	0,98	100,83	6,62E-05	112,13
Slc26a11	1,68E-06	68,61	0,07	107,08	0,01	106,09
Stxbp1	4,36E-07	68,62	0,00	108,28	0,00	92,18
Hook2	8,68E-07	68,77	2,20E-16	143,06	0,65	101,04
6030419C18Rik	3,82E-07	69,00	0,74	98,46	0,01	93,50
Cdh15	6,29E-07	69,01	0,00	107,96	0,42	101,93
Pi4k2b	2,32E-07	69,22	0,45	98,22	1,19E-07	111,81
Skor1	6,04E-07	69,23	0,61	101,54	0,01	93,59
Runx1t1	3,13E-06	69,47	0,97	99,53	4,39E-05	91,55
Rin1	8,89E-07	70,21	0,24	96,03	3,23E-15	125,35
Ccdc104	1,29E-05	70,33	8,52E-05	88,70	3,35E-06	114,59
Fgf15	3,60E-06	70,93	0,69	100,46	2,12E-05	89,07
Col8a1	7,17E-06	70,96	0,14	95,64	0,78	100,54
Plk2	4,31E-06	71,02	0,00	92,16	0,11	103,68
Ppt2	2,98E-06	71,04	0,00	93,22	0,00	108,02
Mcam	2,34E-06	71,08	4,20E-06	110,29	0,00	93,40
Naca	1,42E-05	71,12	0,24	103,05	0,00	89,68
Chchd7	6,73E-06	71,20	3,41E-06	89,78	0,48	97,96
Egfr	3,54E-06	71,21	0,46	98,36	0,01	105,14
Cxcr7	2,46E-06	71,58	0,44	101,16	0,00	105,50
A2m	9,62E-06	71,69	0,04	95,46	0,00	106,53
Gja4	3,88E-05	71,81	0,75	103,28	2,01E-05	89,98
Fam111a	2,25E-05	71,87	0,48	97,97	0,83	100,31
Syp	3,83E-06	71,89	0,25	103,19	3,43E-13	119,78
Stag2	5,85E-06	72,02	4,21E-09	85,46	0,14	103,32
Psmc2	3,09E-05	72,05	0,03	96,53	0,00	93,66
8430408G22Rik	1,60E-05	72,08	0,14	103,00	0,15	96,73
2310043J07Rik	5,77E-06	72,14	0,00	92,20	0,60	101,36
Ror2	3,20E-05	72,23	0,00	109,23	0,00	93,19
Acads	6,88E-05	72,25	0,00	91,41	0,00	109,84
Armxc6	3,49E-05	72,30	0,66	99,13	0,03	105,83
Nif3l1	0,00	72,52	1,35E-05	89,40	0,02	100,01
Mpg	9,30E-06	72,61	0,01	94,91	0,00	108,37
Dido1	3,33E-05	72,69	0,23	103,46	7,03E-05	107,87
Nckap1	7,67E-06	72,70	0,23	97,18	2,20E-16	130,30
Klc4	1,60E-05	72,86	0,02	104,36	3,72E-05	87,60
Gata4	1,24E-05	73,14	0,18	102,49	0,01	93,53
Akr1c12	0,00	73,39	3,58E-05	90,11	0,01	105,24
Marveld2	1,66E-05	73,40	1,33E-11	125,18	2,20E-16	138,46
Aldoc	5,43E-05	73,42	0,52	101,75	0,65	101,06
Kank2	6,92E-05	73,70	0,05	105,10	5,52E-07	86,51
I14ra	2,30E-07	73,74	0,00	106,98	0,04	96,08
Col6a2	4,23E-05	73,75	0,00	110,30	2,51E-08	87,24
Arhgef7	5,11E-05	73,80	0,01	93,60	0,08	103,46
Ap2m1	6,34E-05	73,83	0,09	96,28	1,69E-07	115,15
Fbl	9,77E-05	73,84	0,05	95,59	0,00	107,64
Cdc25b	0,00	73,92	0,77	103,15	7,43E-07	88,43
Fus	4,89E-05	74,05	0,00	106,34	1,25E-06	109,31
Ccl2	7,51E-06	74,51	0,25	96,21	0,95	99,76
Exoc3l	0,00	74,92	3,35E-05	109,53	0,76	98,97
Rnf123	7,14E-05	75,05	0,64	100,97	4,26E-09	114,08
Rcc2	0,00	75,34	1,66E-10	117,21	0,05	96,34
Sphk1	0,00	75,56	9,54E-10	117,15	2,19E-09	86,44
Lrtm2	0,00	75,62	0,41	101,97	0,47	102,04
Scp2	0,00	76,24	9,34E-16	76,99	3,53E-14	121,93
Rtn4ip1	0,00	76,30	0,84	100,23	0,80	100,70
Dicer1	0,00	76,49	0,99	99,40	3,12E-08	111,89
Arap3	0,00	76,54	0,00	93,38	0,00	106,59

Tmem63a	0,00	76,61	0,97	99,30	0,88	99,14
Scaf1	0,00	77,13	0,01	108,58	0,00	105,95
Zfp105	0,00	77,29	0,28	102,98	1,93E-06	112,93
Rtn2	0,00	77,31	0,26	95,97	0,00	108,61
Pdcd4	0,00	77,49	0,01	106,45	4,05E-08	113,10
Sox15	0,00	77,58	0,00	92,23	0,02	104,41
Fermt3	0,00	79,35	7,73E-05	111,41	0,02	104,79
4930563D23Rik	0,00	82,53	0,00	93,65	4,47E-09	118,02

Supplementary Table 2

Scores (as normalized to 100 to the effect of negative controls, RLuc and FLuc), accompanied with their respective P-values, obtained after esiRNA transfection of the indicated genes into the mouse myogenic cell line C2C12. Effects on fusion (nuclei per fibre, in red), proliferation/cell survival (nuclei count, in blue) and myogenic differentiation (myogenin expression, in green) after loss of function of those molecules are indicated. The list encompasses the Entrez gene IDs of the esiRNAs that obtained the lowest score in the fusion assay. The corresponding genes are therefore predicted to act as most necessary for C2C12 fusion.

Table 3. Known fusion genes (with references) tested in the esiRNA screen

Gene	Modulate C2C12 fusion (esiRNA test)	References	Remark
<i>Anxa1</i>	No	Leikina et al. J Cell Biol 2013 ¹	Anxa1 & a5 act redundantly
<i>Anxa5</i>	No	Leikina et al. J Cell Biol 2013 ¹	
<i>Arhgap17</i>	Yes	Doherty et al. J Biol Chem 2011 ²	Anxa1 & a5 act redundantly
<i>Arhgap23</i>	Yes	Doherty et al. J Biol Chem 2011 ²	
<i>Arhgap30</i>	Yes	Doherty et al. J Biol Chem 2011 ²	
<i>Bai3</i>	No	Hamoud et al. Nature Comm 2018 ³	
<i>Capn7</i>	Yes	Buffolo et al. Genesis 2015 ⁴	
<i>Cdc42</i>	Yes	Vasyutina et al. PNAS 2009 ⁵	
<i>Cdh15/m-Cadh</i>	Yes	Charrasse et al. Mol Cell Biol 2007 ⁶	
<i>Ciql3</i>	Yes	Hamoud et al. Nature Comm 2018 ³	
<i>Dock1</i>	NT	Laurin et al. PNAS 2008 ⁷	
<i>Fhl1</i>	Yes	Cowling et al. J Cell Biol 2008 ⁸	
<i>Graf1</i>	NT	Lenhart et al. Dev Biol 2014 ⁹	
<i>Il4ra</i>	Yes	Horsley et al. Cell 2003 ¹⁰	
<i>Itga6</i>	Yes	Wilschut et al. Stem Cell Research 2011 ¹¹	
<i>Itgb1</i>	NT	Schwander et al. Dev Cell 2003 ¹²	
<i>Jam2</i>	No	Powell and Wright Plos Biol 2011 ¹³	Jam2 & 3 act redundantly
<i>Jam3</i>	NT	Powell and Wright Plos Biol 2011 ¹³	Jam2 & 3 act redundantly
<i>Mapk7/Erk5</i>	NT	Sunadome et al. Dev Cell 2011 ¹⁴	
<i>Myd88</i>	Yes	Hindi et al. Nature Comm 2017 ¹⁵	
<i>Mymk</i>	NT	Millay et al. Nature 2013 ¹⁶	
<i>Mymx</i>	NT	Quinn et al. Nature Comm 2017; Bi et al. Science 2017; Zhang et al. Nature Comm 2017 ^{17,18,19}	
<i>Myof</i>	No	Doherty et al. Development 2005 ²⁰	
<i>Neogenin</i>	NT	Bae et al. Mol Biol Cell 2009 ²¹	
<i>Nfatc2</i>	Yes	Horsley et al. Cell 2003 ¹⁰	
<i>Nphs1/Nephrin</i>	NT	Sohn et al. PNAS 2009 ²²	
<i>Npnt</i>	Yes	Lee et al. Sci Rep 2017 ²³	
<i>Nr2f2/Coup TFII</i>	Yes	Lee et al. Sci Rep 2017 ²³	
<i>Pak1</i>	Yes	Duan et al. J Cell Biol 2012 ²⁴	
<i>Prkcq</i>	Yes	Madaro et al. Mol Biol Cell 2011 ²⁵	
<i>Ptk2/Fak</i>	NT	Quach et al. Mol Biol Cell 2009 ²⁶	
<i>Rac1</i>	Yes	Vasyutina et al. PNAS 2009 ⁵	
<i>RhoA</i>	NT	Wei et al. JBC, 1998 ²⁷	
<i>Stab2</i>	No	Park et al. Nature Comm 2016 ²⁸	
<i>Wasl/N-Wasp</i>	Yes	Gruenbaum-Cohen et al. PNAS 2012 ²⁹	

No: No effect on C2C12 fusion
Yes: Effect on C2C12 fusion
NT: Not tested

Supplementary Table 3

List of genes known from the literature to modulate fusion (with references) and that were tested or not in the esiRNA screen in the present study.

Table 3. Role of TGF β -associated genes on C2C12 fusion

TGF β ligands (TGFBs, INHs, MST, NODAL)	Fusion (% of Ctrl)	Proliferation	Differentiation (MyoG)
TGFB1	ns		
TGFB2	406	112	154
TGFB3	198	97	118
INHA	ns		
INHBC	ns		
INHBE	ns		
FST (Myostatin inhibitor)	ns		
TGFβ receptor			
TGFB1	284	97	149
TGFB3 (co-receptor)	183	106	120
ACVR1B	254	112	124
ACVRL1	ns		
ACVR2B (MST unique receptor II)	ns		
ACVR2A	ns		
Effectors			
SMAD2	166	91	131
SMAD3	378	100	137
SMAD4	524	98	157
Synergizes with TGFβ^(ref)			
DPT ⁽³⁰⁾	148	113	113
MMP14 ⁽³¹⁾	154	90	126
RUNX1 ⁽³²⁾	179	107	125
SCUBE3 ⁽³³⁾	220	108	132
TGFβ antagonists			
TGIF1	57	88	79
BMP pathway			
BMPR1A	ns		
BMP 1, 4, 7, 8	ns		
ACVR2A, 2B	ns		
SMAD5	ns		
Positive controls			
RAC1	73	85	142
CDC42	52	81	140

ns: non significant

note: TGFB1,2,3 bind TGFB1 or ACVRL1 (type I receptors) and only bind TGFB2 (type II receptors)

MST binds TGFB1 or ACVR1B (type I receptors) and only binds ACVR2B (type II receptor)

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