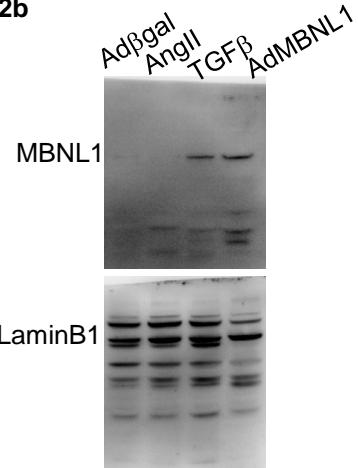
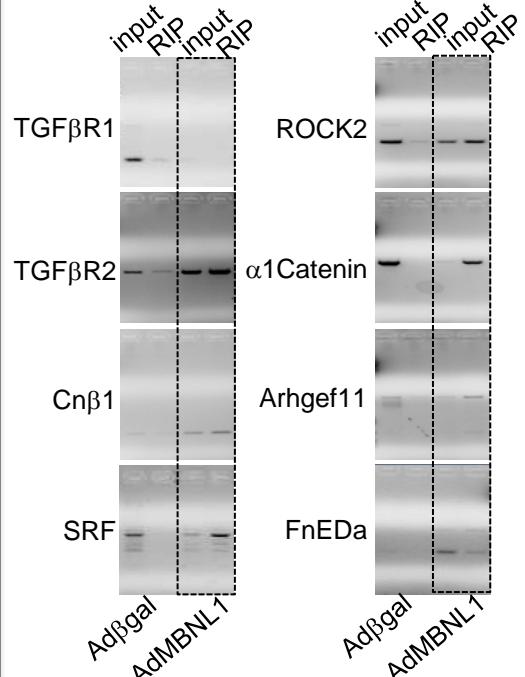


**Supplementary Figure 1.** Full length images of the agarose gels and Western blots presented in the manuscript.

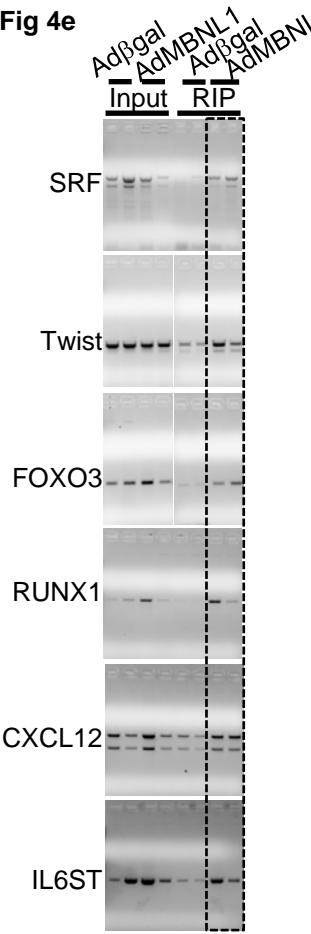
**Fig 2b**



**Fig 4d**

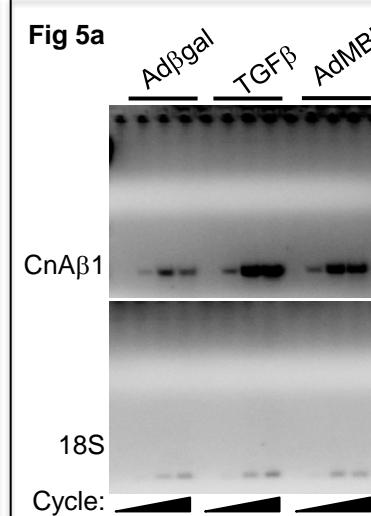


**Fig 4e**

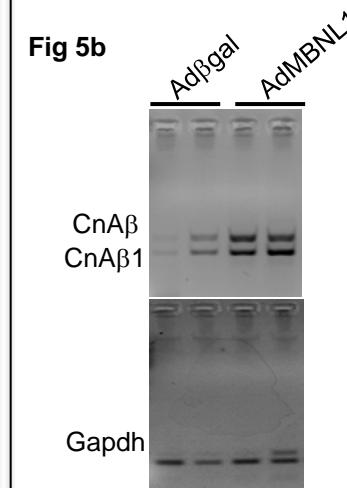


**Fig 5a**

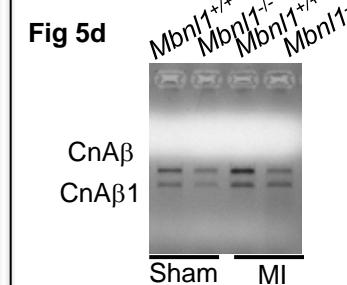
**Fig 5a**



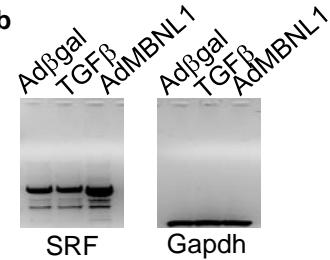
**Fig 5b**



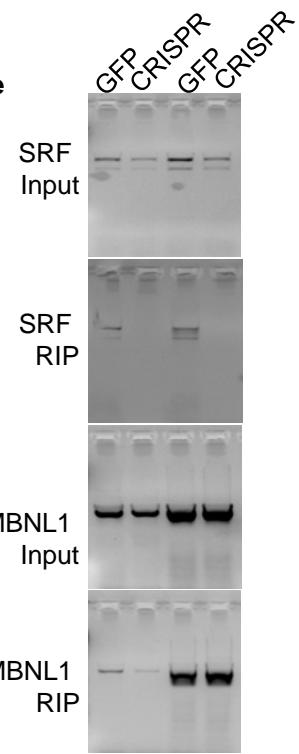
**Fig 5d**



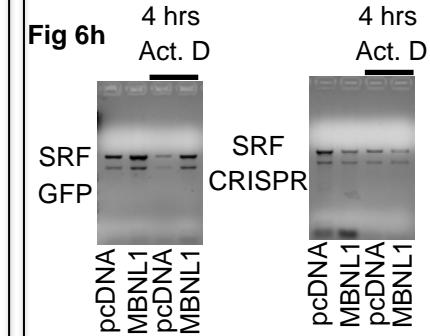
**Fig 6b**

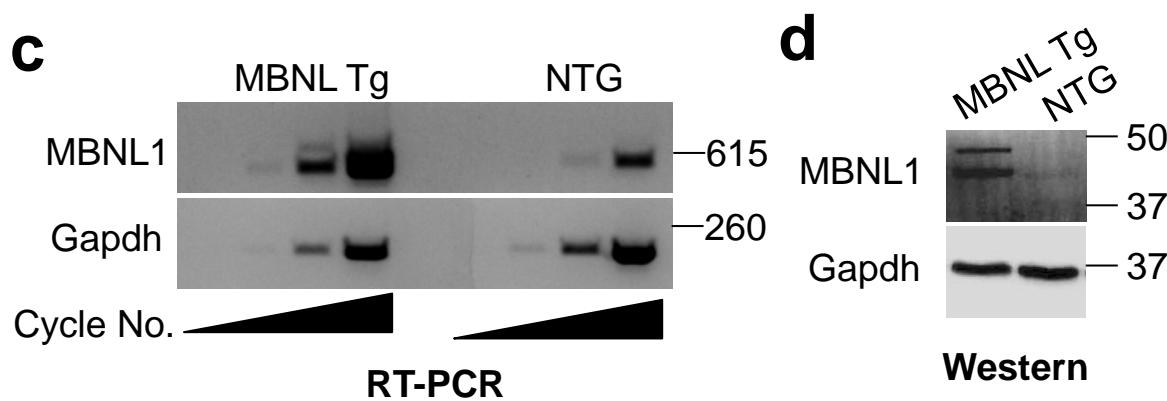
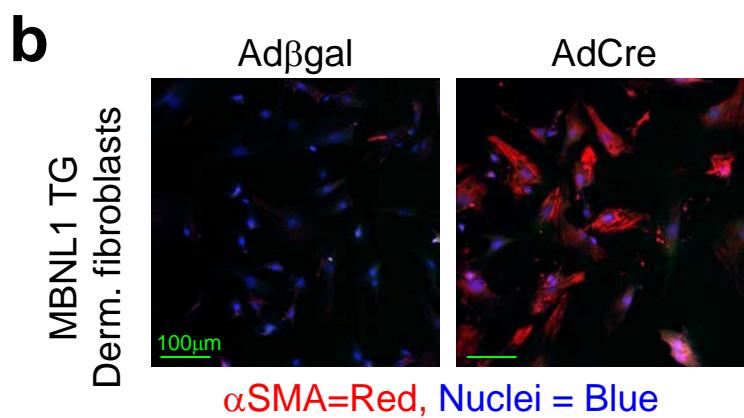
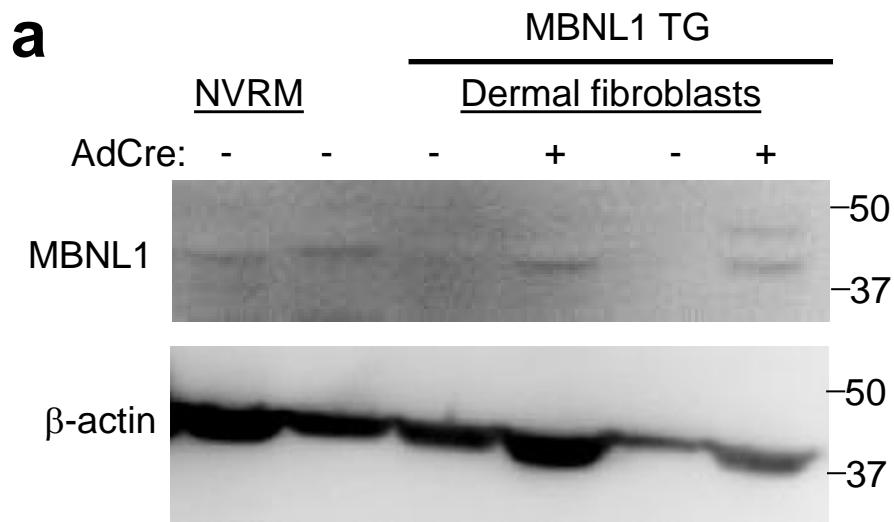


**Fig 6e**

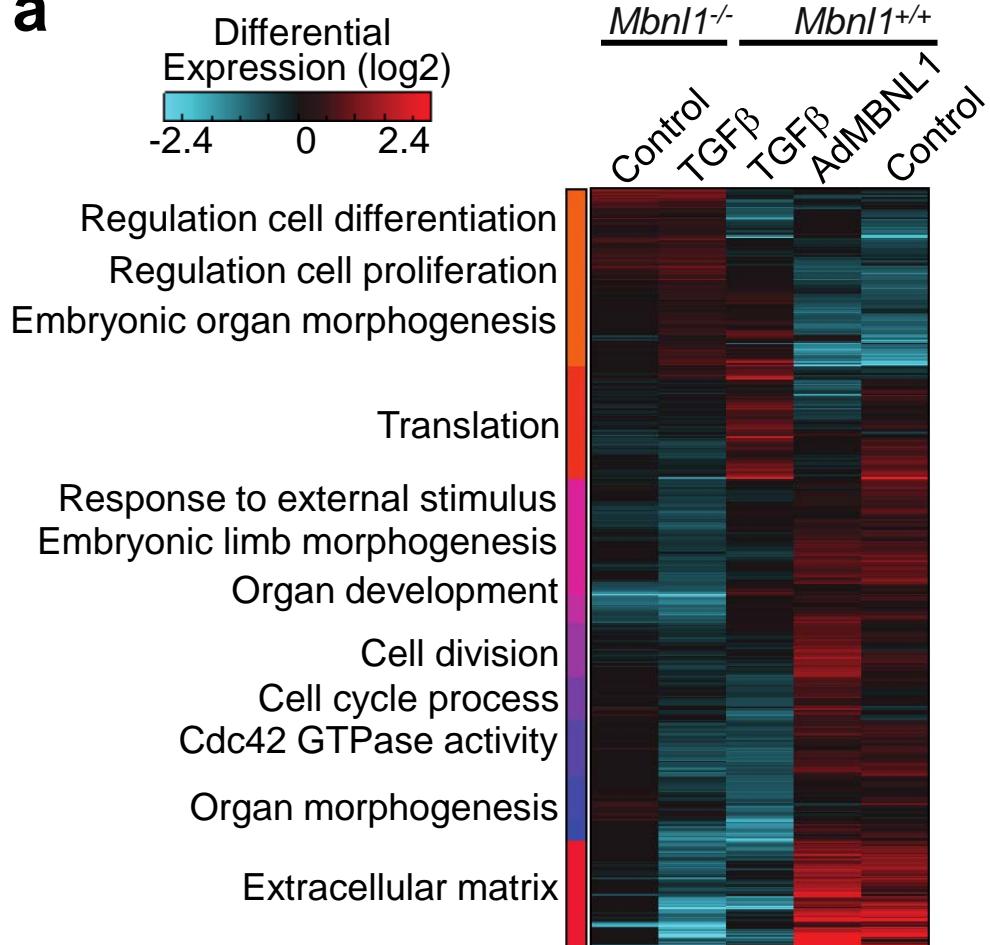
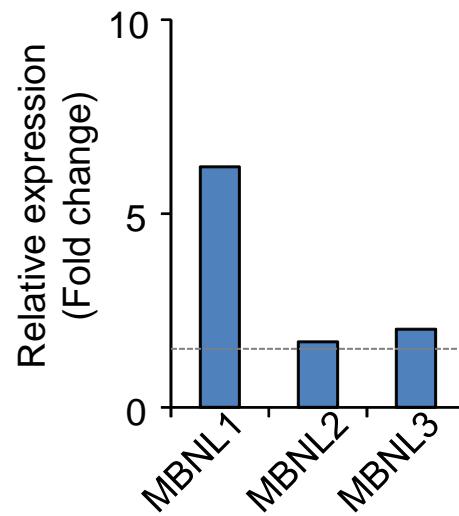
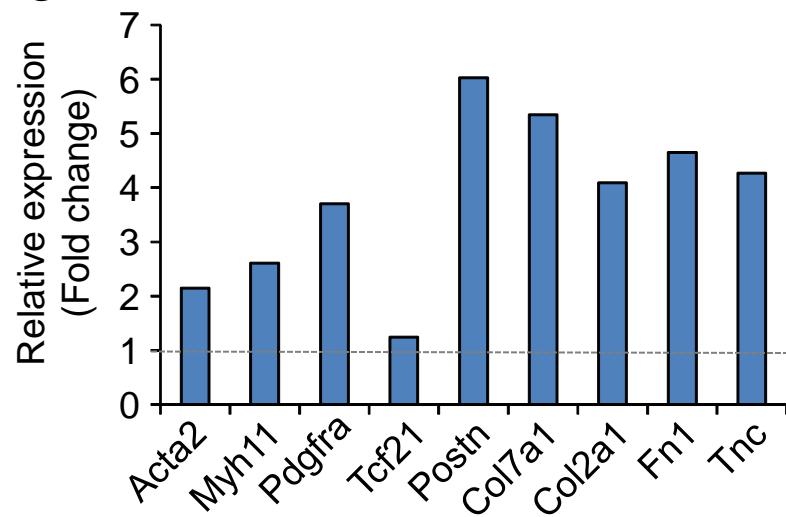


**Fig 6h**

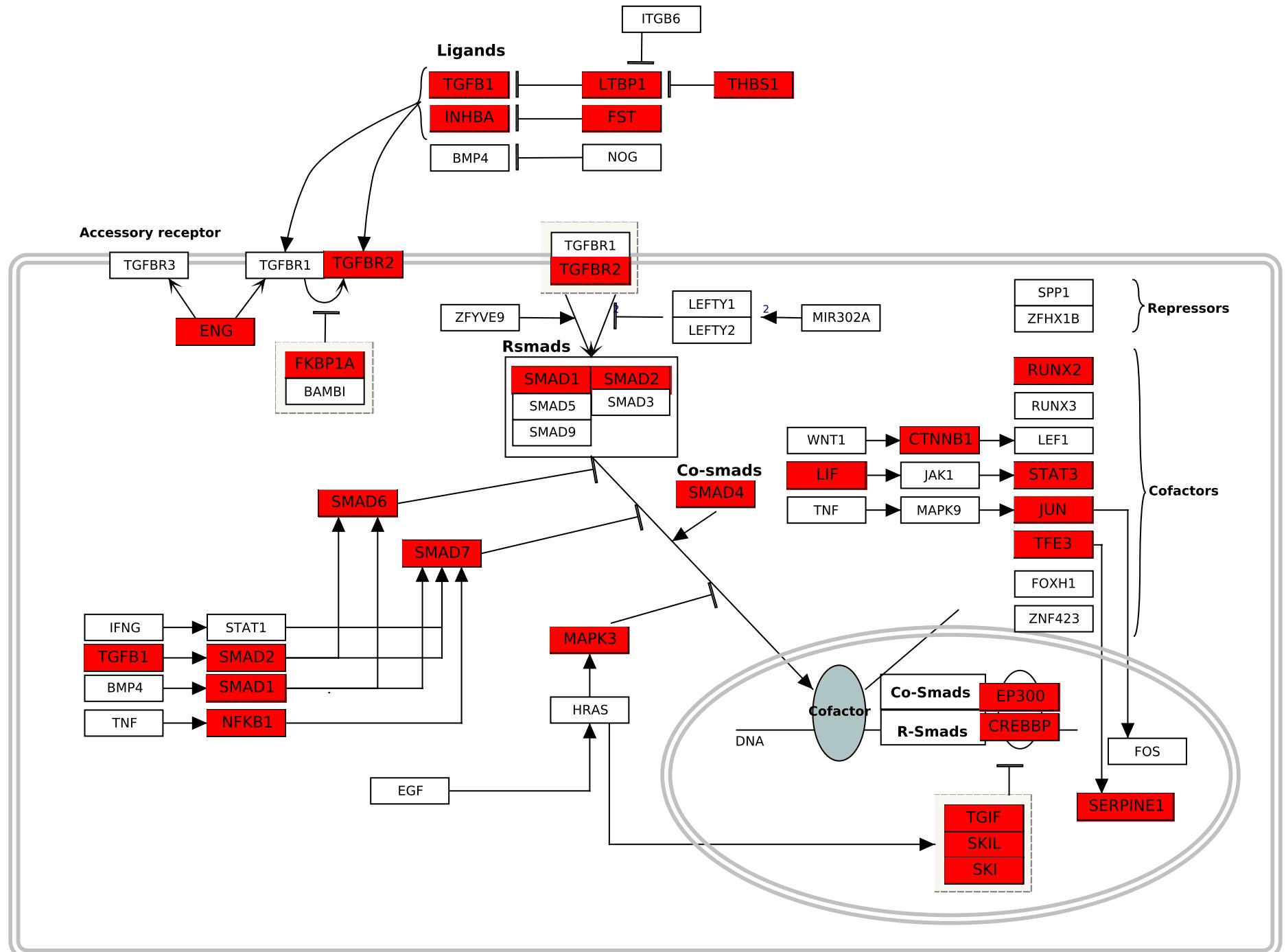




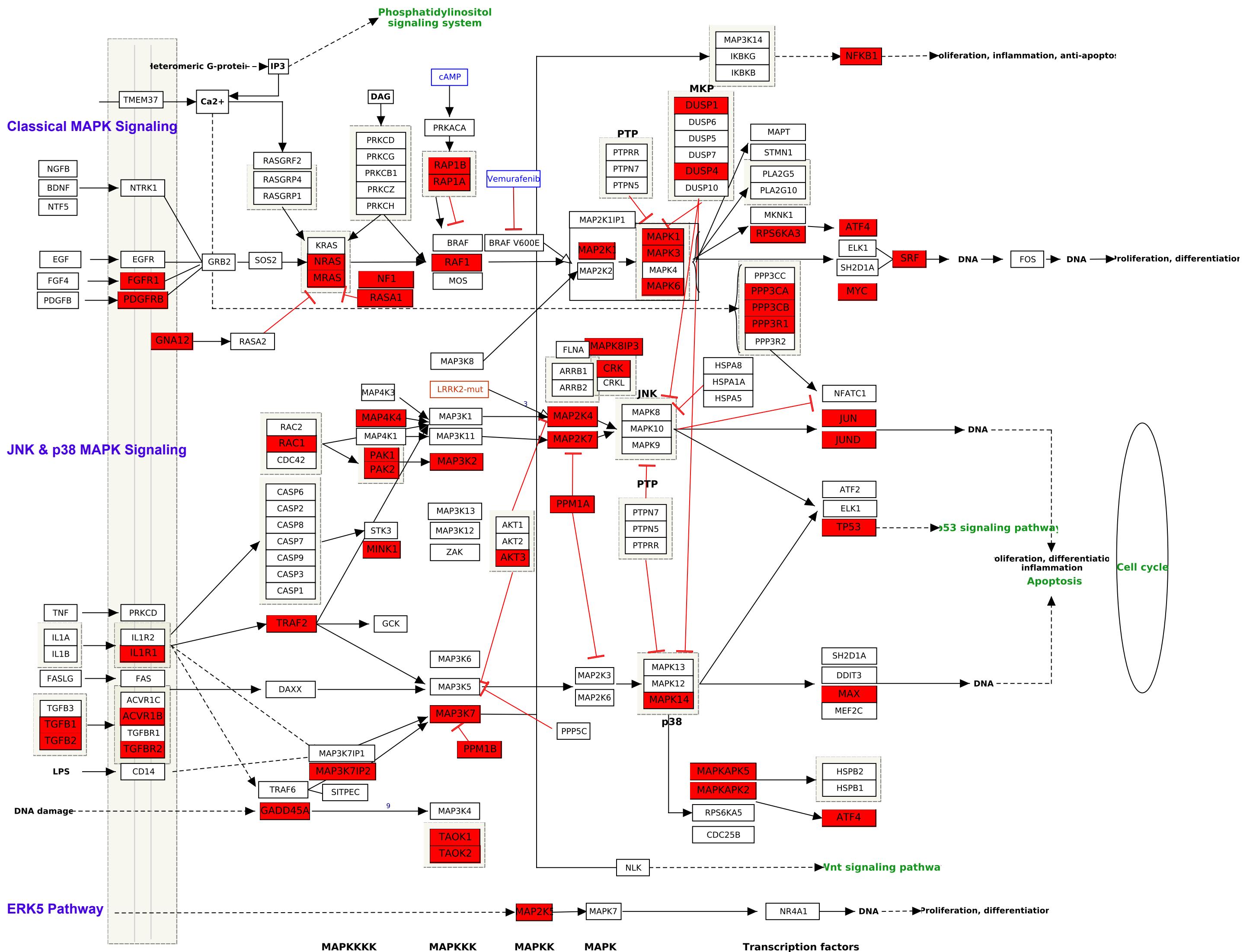
**Supplementary Figure 2.** Analysis of Mbnl1 expression in fibroblasts derived from Mbnl1 transgenic mice. **(a)** Western blot analysis of Mbnl1 expression in primary dermal fibroblasts isolated from LoxP-dependent Mbnl1 TG mice adenovirally infected with either AdCre or Ad $\beta$ gal (-). Neonatal rat cardiac myocytes (NVRM) were used as a positive control for Mbnl1 expression.  $\beta$ -actin was used as a loading control. **(b)** Immunofluorescent staining of  $\alpha$ SMA-positive (red) stress fibers in primary dermal fibroblasts isolated from LoxP-dependent Mbnl1 TG mice adenovirally infected with either AdCre or Ad $\beta$ gal. Scale bar = 30  $\mu$ m. **(c)** RT-PCR analysis of Mbnl1 mRNA in fibroblasts isolated from the hearts of Mbnl1 TG- $Tcf21^{MCM}$  and NTG- $Tcf21^{MCM}$  mice. Gapdh was used as a control. **(d)** Western blot analysis of Mbnl1 expression in fibroblasts isolated from the hearts of Mbnl1 TG- $Tcf21^{MCM}$  and NTG- $Tcf21^{MCM}$  mice. Gapdh was used as a loading control. Molecular weight markings are shown on the right of the blots in panels a and d, while DNA size in base pairs is marked on the right of panels in c. Refers to [Figure 3](#)

**a****b****c**

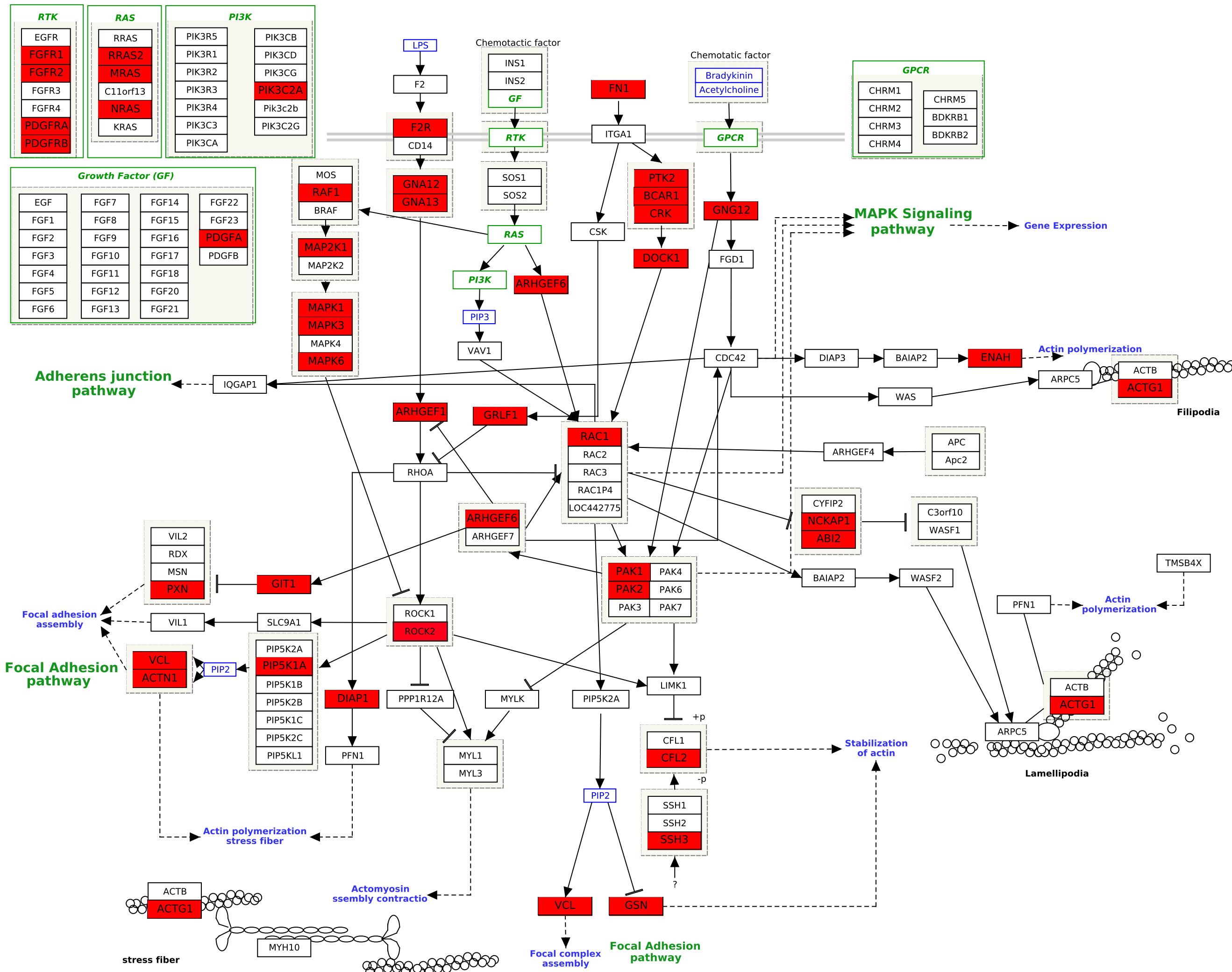
**Supplementary Figure 3.** MBNL1 transduced MEFs have the genetic profile of a fully matured myofibroblast. **(a)** Heat map depicting differential gene expression clusters generated from RNAseq in *Mbnl1<sup>-/-</sup>* and *Mbnl1<sup>+/+</sup>* MEFs with and without TGF $\beta$  or AdMBNL1 infection. The list on the left corresponds to changed gene categories in the heat map. **(b)** RNA expression levels of the MBNL gene family (MBNL1-3) in MEFs infected with AdMBNL1. **(c)** Key myofibroblast genes in MEFs infected with AdMBNL1, expressed relative to control, as determined by RNAseq analysis. Refers to [Figure 4](#).



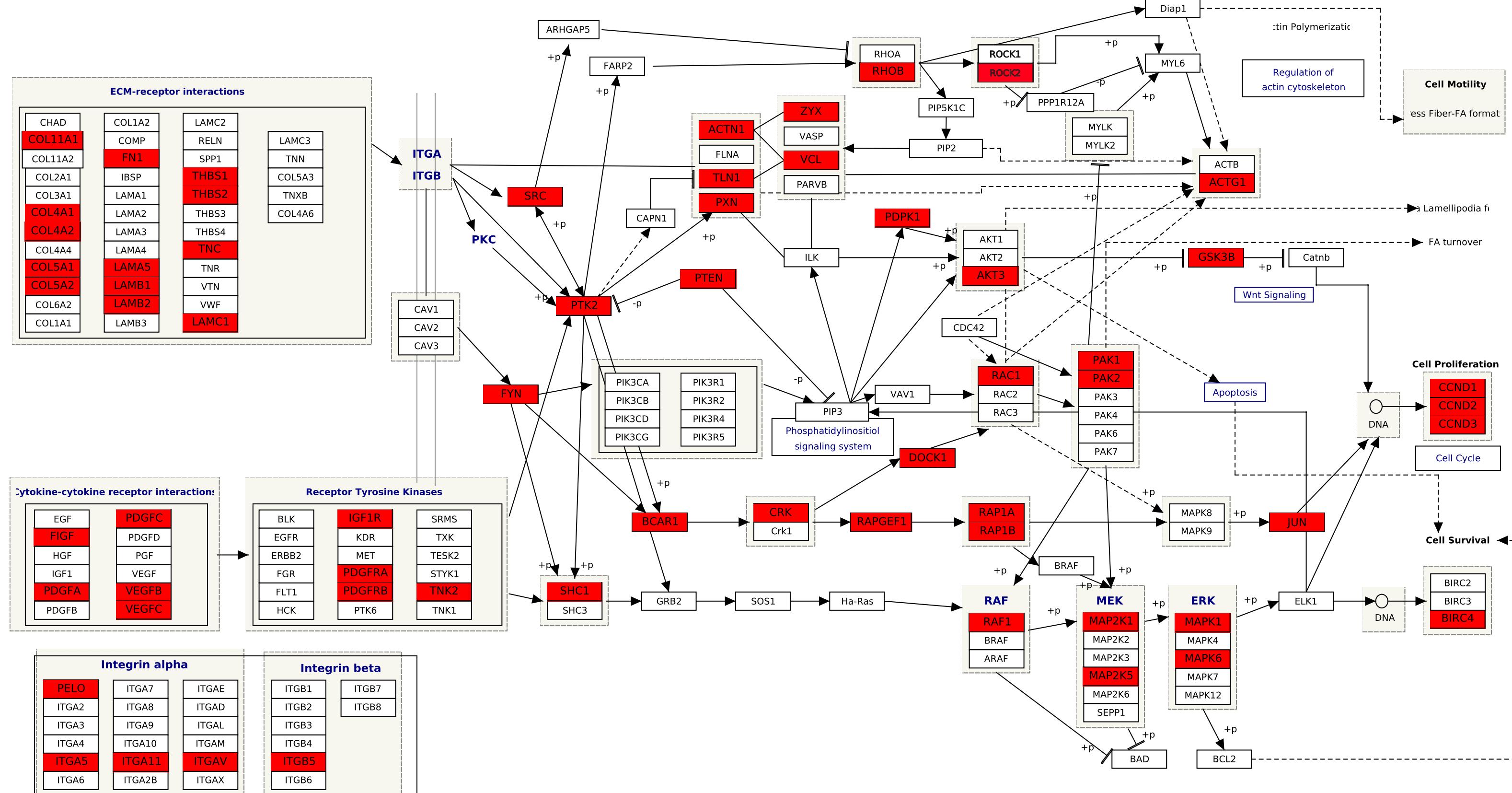
**Supplementary Figure 4.** Wikipathway for TGF $\beta$  signaling (WP560). Transcripts that immunoprecipitated with MBNL1 are colored in red. Refers to [Figure 4](#)



Supplementary Figure 5. Wikipathway for MAPK signaling (WP382). Transcripts that immunoprecipitated with MBNL1 are colored in red. Refers to Figure 4



**Supplementary Figure 6** Wikopathway for the regulation of the actin cytoskeleton (WP51). Transcripts that immunoprecipitated with MBNL1 are colored in red. Refers to [Figure 4](#)



**Supplementary Figure 7** Wikipathway for focal adhesion signaling (WP360). Transcripts that immunoprecipitated with MBNL1 are colored in red. Refers to [Figure 4](#)

**Supplementary Table 1. MBNL1 RIP targets with identified or predicted conserved binding sites, Related to Figure 4.**

A list of MBNL1-bound RNAs (MBNL1 RIP-Seq) categorized by predicted 3'UTR conserved binding sites, CLIP-seq identified 3'UTR binding sites, CLIP-seq identified intronic binding sites, and CLIP-seq identified exonic binding sites. Blue text indicates targets confirmed in Figure 4

RIP Targets with Conserved 3' UTR Binding Sites (Human Sequence)	RIP Targets with Identified 3'UTR Binding Sites (CLIP-Seq)	RIP Targets with Identified Intronic Binding Sites (CLIP-Seq)	RIP Targets with Identified Extrinsic Binding Sites (CLIP-Seq)
ABHD2	Abi1	Ankrd13a	Acap2
ACVR1	Actc1	Atp2a2	Akt3
ADAMTS1	Actr2	Capza1	Atf6
ADM	Adam10	Dst	Cxadr
AGO2	Aes	Dynll2	Dst
AGTR1	Aff4	Eif5	Enah
ALDH3A2	Akap2	Evl	Lamp2
ALKBH5	Alkbh5	Ext1	Lats2
ANG	Ammecr1l	Fam168a	Luc7l2
ANTXR2	Aplp2	Galnt2	Msi2
AP3S2	Arhgef11	Grb14	Nfia
ARL6IP5	Arih1	Mef2a	Nptn
ATP6AP2	Atp1b1	Pigf	Pdlim5
AXL	Atp1b3	Plekhb2	Plcg1
B4GALT5	Atp2a2	Rmnd5a	Ppp1cb
BCKDHA	Azin1	Sestd1	Rab24
BGN	Bcl7b	Slc39a14	Rcn2
BMP6	Bmpr1a	Snx12	Sdcbp
BMPR2	Cacna2d1	Spata5	Snx12
BNIP3	Camk2d	Tial1	Tnrc18
BUB3	Cfbf	Trak1	Trak2
C1R	Cbx6	Zmiz1	Usp9x
C1S	Cds2		Wapal
C2	Celf1		Yap1
C3	Celf2		Sod2
CADM1	Cggbp1		
CARHSP1	Cited2		

CBFB	Clic4
CCL2	Cmpk1
CCL7	Col4a2
CD151	Crip2
CD200	Csnk1a1
CD55	Ctnnb1
CD59	Dcaf8
CDH2	Degs1
CDKN1A	Dr1
CEBPB	Dync1li2
CEBPD	Dynll2
CELF1	Edem1
CHI3L1	Ehd4
CHPT1	Eif2ak1
CHST11	Eif4e
CITED2	Epc2
CLU	Epdr1
CNPPD1	Fhl2
COL12A1	Fstl1
COL16A1	Gnb1
COL4A1	Golph3
COL4A2	Grk5
COL8A1	Hif1a
COMMD3	Hipk1
CPE	Hipk3
CRIM1	Hnrnpul2
CRLF1	Il6st
CSF1	Inpp5a
CST3	Ivns1abp
CTSH	Klhdc10
<b>CXCL12</b>	Laptm4a
CXCL16	Lman2
CXCL6	Lrrc58
CYR61	Lsm14a
DCN	Mapk1
DDOST	Mapk14
DES	Mat2b
DPAGT1	Max
DPY19L1	Mbnl1
DUSP1	Mbnl2
EDN1	Msi2

EGLN1	Mtpn
EMILIN1	Myadm
ENG	Nampt
ENO2	Ndel1
ENPP2	Nedd4
EPHB4	Nf1
ERAP1	Nfat5
ERO1L	Nploc4
ESYT2	Nudt3
F2R	Ociad1
FAM129B	Otud4
FAM171A1	Oxct1
FBLN1	Pafah1b2
FBXL5	Paip2b
FIGF	Pcmt1
FLRT2	Pdrg1
FMOD	Pls3
<b>FN1</b>	Ppp2r5c
FST	<b>Ppp3cb</b>
FURIN	Ppp3r1
FZD1	Prkacb
GAA	Prkar1a
GABARAPL1	Psap
GABARAPL2	Rab1
GADD45G	Rab10
GALK2	Rab11b
GALNT2	Rab18
GDA	Rab2a
GFPT2	Rap1b
GJA5	Rbfox2
GLB1L	Rbm39
GLIPR1	Rbpms
GLUL	Rftn1
GNS	Rhoq
GPM6B	Rmnd5a
GPR125	Rtn4
GPR176	Serinc3
GREM1	Setd7
GSDMD	Ski
GSTM5	Snx18
GYS1	Snx3

H3F3B	Socs5
H3F3C	Spc83
HIGD1A	Sqstm1
HM13	Stk24
HMOX1	Strn3
HP	Tbl1xr1
HPRT1	Tead1
HPX	Tial1
HS6ST1	Tmem106b
HSPB8	Tmtc1
ICAM1	Tmx4
IGF1R	Tomm70a
IGFBP3	Tsc22d1
IGFBP7	Tsn
IL1R1	Ttc39b
IL1RL1	Tuba4a
IL4R	Ubqln2
IL6ST	Usp14
IMPAD1	Vamp3
IRF2BP2	Vcl
ISG15	Vezf1
ITGA5	Wbp2
ITM2C	Wnk1
JAM3	Ywhag
JUNB	Zbtb44
KHDRBS1	Zfp106
KLHL24	
LAGE3	
LAMA5	
LAMB1	
LAMP2	
LAPTM4A	
LDHA	
LGALS3BP	
LGALS9	
LMO4	
LOX	
LRP1	
LRP10	
LTBP1	
LTBP2	

LTBP3

LY6E

LYPLA2

MAN2B1

MAOA

**MBNL1**

MGAT1

MGP

MIF

MMP2

MRC2

MT2A

MX2

NDFIP2

NDUFA2

NFIB

NFKBIA

NOV

NT5E

OLR1

ORMDL3

OSMR

OST4

P4HA2

PALLD

PAM16

PARM1

PBX1

PDGFRA

PDRG1

PELI1

PFKL

PGRMC1

PGRMC2

PLA1A

PLBD2

PLEKHA3

PLOD2

PML

PNRC1

PPAP2A

PRELP  
PRNP  
PROS1  
PRRX1  
PSEN2  
PSMF1  
PTBP3  
PTGIS  
PTK7  
PTX3  
RAB32  
RANBP9  
RASL11B  
RBP1  
RBPM5  
RDM1  
RETSAT  
RGS16  
RGS2  
RNASE4  
RPP14  
SDC4  
SELT  
SEMA3C  
SERINC1  
SERPINB9  
SERPINE1  
SERPING1  
SHISA5  
SLC2A1  
SLC2A3  
SLC35B1  
SLC39A14  
SLC7A2  
SLIT3  
SMAD7  
SMIM14  
SMIM7  
SMOC1  
SMPD1  
SOD2

SOD3  
SSR3  
STAT2  
SULF1  
SUN2  
TAGLN2  
TEAD1  
TF  
TFPI2  
TGM2  
THBS2  
TIMP1  
TMEM167B  
TMEM176A  
TMEM179B  
TMEM87A  
TNC  
TNPO2  
TOMM6  
TPI1  
TPP1  
TSPAN31  
TSPAN6  
TSPO  
UAP1  
UBALD2  
UBC  
UBR4  
UGCG  
UGDH  
VCAN  
VEGFC  
VGLL3  
VMP1  
WDFY3  
WFDC1  
WIPI2  
YPEL3  
ZFAND5  
ZFP36L1  
ZNRF2

**Supplementary Table 2.** Fold enrichment of MBNL1 bound messages, refers to [Figure 4](#)

Transcript	Input (%)	Fold Enrichment ( $2^{-\Delta Ct}$ )
<b>TGF<math>\beta</math>R1</b>	<b>0.24</b>	<b>0.00</b>
<i>stdev</i>	0.07	0.00
<b>TGF<math>\beta</math>R2</b>	<b>0.19</b>	<b>0.66</b>
<i>stdev</i>	0.02	0.05
<b>CnA<math>\beta</math>1</b>	<b>4.10</b>	<b>0.45</b>
<i>stdev</i>	1.89	0.05
<b>SRF</b>	<b>1.41</b>	<b>0.76</b>
<i>stdev</i>	0.42	0.21
<b>ROCK2</b>	<b>0.49</b>	<b>2.26</b>
<i>stdev</i>	0.15	1.13
<b><math>\alpha</math>1CATENIN</b>	<b>3.16</b>	<b>25.59</b>
<i>stdev</i>	2.01	12.60
<b>ARHGEF11</b>	<b>1.32</b>	<b>47.77</b>
<i>stdev</i>	0.20	23.70
<b>TWIST1</b>	<b>1.83</b>	<b>0.66</b>
<i>stdev</i>	1.74	0.22
<b>FOXO3</b>	<b>0.21</b>	<b>2.54</b>
<i>stdev</i>	0.05	1.28
<b>RUNX1</b>	<b>0.28</b>	<b>5.59</b>
<i>stdev</i>	0.08	2.60
<b>CXCL2</b>	<b>0.24</b>	<b>1.27</b>
<i>stdev</i>	0.08	0.68
<b>IL6ST</b>	<b>0.29</b>	<b>4.71</b>
<i>stdev</i>	0.04	2.36
<b>FnEDa</b>	<b>0.24</b>	<b>0.20</b>
<i>stdev</i>	0.10	0.10