Supplemental Materials

Molecular Biology of the Cell

Patrinostro et al.

Supplemental Methods

Primers

Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')
mACTA1 Cloning	CACCTCACTTCCTACCCTCGGCA	GCAAAACAGAATGGCTGGCTT
mACTA2 Cloning	CACCGCTGCTCCAGCTATGTGTGA	ATCCATGCACGTGTGTATGCTTTA
mACTB Cloning	CACCGCGAGCACAGCTTCTTTG	AGGTAAGGTGTGCACTTTTATTGGT
mACTC Cloning	CACCCGCCTACAGAACCCACCAAA	TGCAAGTCCTGGTCTGGTTTA
mACTG1 Cloning	CACCCCGCCGCCGGCTTACAC	CAGTTACTGCAGCACTTTTATTTC
mACTG2 Cloning	CACCTTGCTCTGGTATTTCTGCCAA	GGTTTTAATGATCTGTGGCTGGTC
Note: CACC is added to all forward cloning primers in order to facilitate directional cloning into pDENTR vector, they are not part of the actin gene sequence.		
mACTA1 qRT	GCCAGCCTCTGAAACTAGACA	CCCACGATGGATGGGAACAC
mACTA2 qRT	TCCAGCTATGTGTGAAGAGGA	CCAACCATTACTCCCTGATGTCT
mACTB qRT	GCGAGCACAGCTTCTTTG	TTTGCACATGCCGGAGCCGTT
mACTC qRT	GATGTGTGACGACGAGGAGA	ATACCCACCATGACACCCTGG
mACTG1 qRT	GATTAAGATCATTGCTCCCCCTGA	GCACCTGCTCAGTCCATCTA
mACTG2 qRT	ATGTGTGAAGAAGAGACCACCG	GCCCATTCCCACCATCACAC

ON-TARGET Plus siRNA Sequences

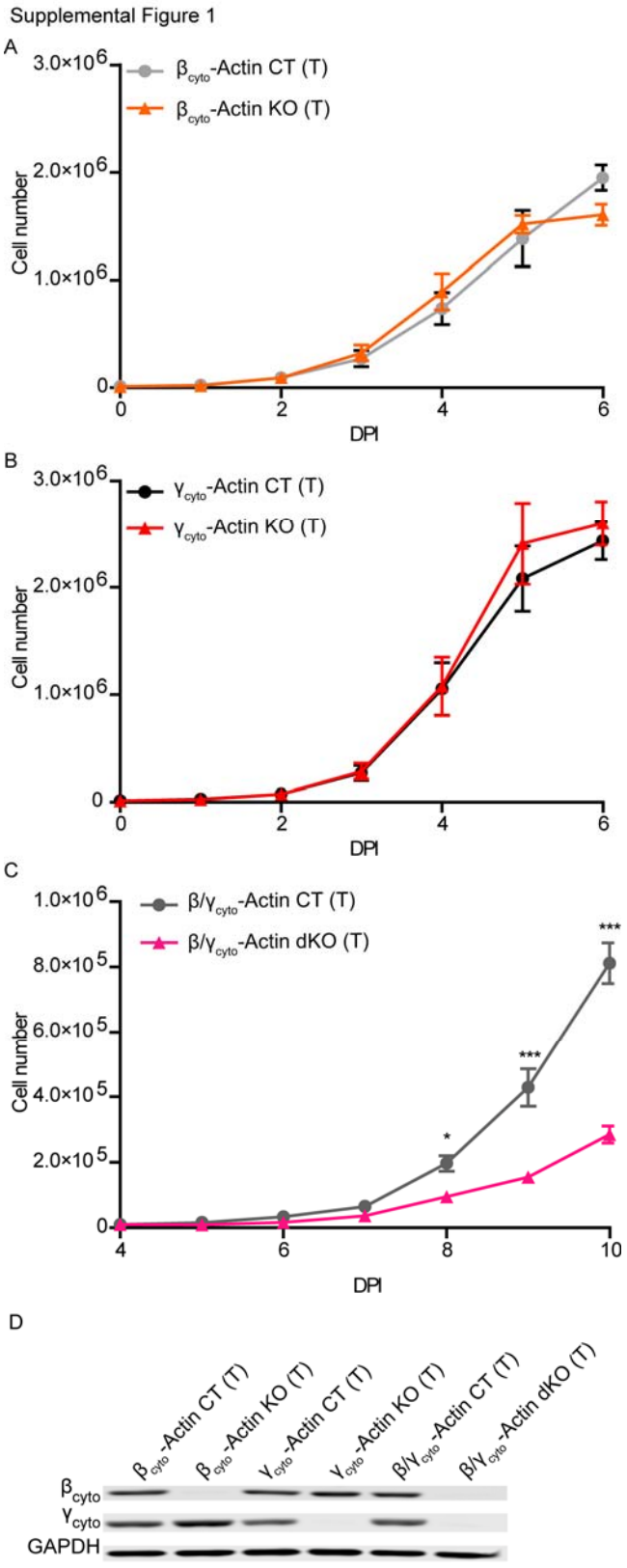
Control ON-TARGETplus Non-targeting Pool Cat# D-001810-10-05	5'-UGGUUUACAUGUCGACUAA-3' 5'-UGGUUUACAUGUUGUGUGA-3' 5'-UGGUUUACAUGUUUUCUGA-3' 5'-UGGUUUACAUGUUUUCUA-3'
βcyto-actin ON-TARGETplus Mouse Actb siRNA-SMARTpool Cat# L-057827-01-0005	5'-GCAAGUGCUUCUAGGCGGA-3' 5'-UUUUAAAUCUUCCGCCUUA-3' 5'-CCAAGUAUCCAUGAAAUAA-3' 5'-GCAGUUGGUUGGAGCAAAC-3'
γcyto-actin ON-TARGETplus Mouse Actg1 siRNA-SMARTpool Cat# L-042869-00-0005	5'-ACGCAGAUAAUGUUUGAAA-3' 5'-CCUAGCACGAUGAAGAUUA-3' 5'-AGAGGGAAAUUGUUCGUGA-3' 5'-GCAUGGAGUCCUGUGGUAU-3'

Supplemental table 1

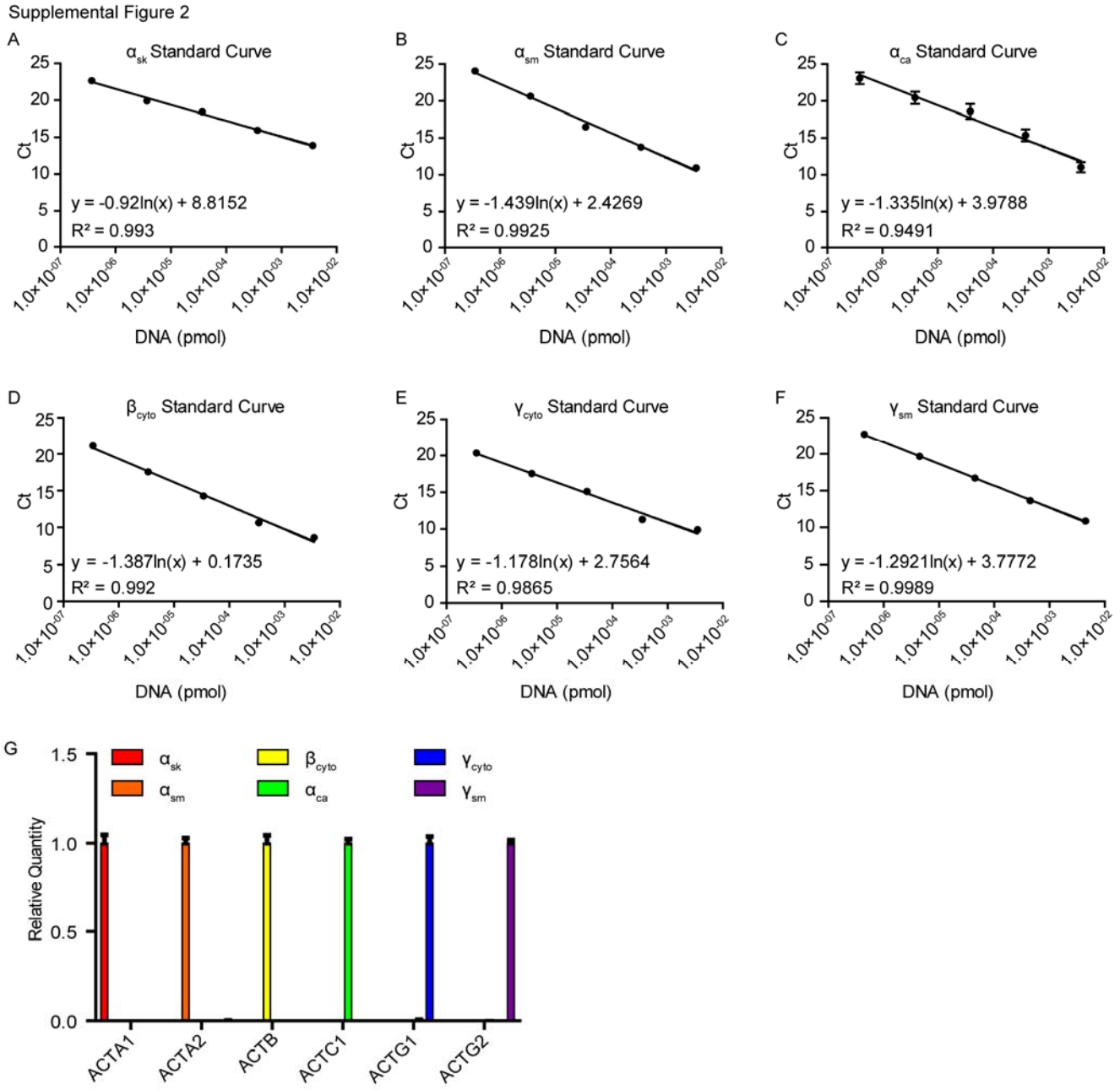
Mouse Actin Isoform	Target Scan	microRNA.org	PicTar	Consensus miRNA
ACTB	mir-1 mir-132 mir-145 mir-205 mir-206 mir-212 mir-212-3p mir-34a mir-34b mir-194 mir-613	mir-1 mir-1192 mir-1295p mir-132 mir-145 mir-194 mir-205 mir-206 mir-212 mir-495 mir-544 let-7a let-7b	mir-124a mir-145 mir-18a mir-18b mir-205	mir-145 mir-205
ACTG1	mir-103a mir-107 mir-10a mir-10b mir-10c mir-145 mir-31 mir-485	mir-103 mir-107 mir-10a mir-10b mir-145 mir-186 mir-199a mir-31 mir-361 mir-381 mir-615 mir-873	mir-10a mir-10b mir-145 mir-199a	mir-10a mir-10b mir-145

Supplemental table 1: Predicted Actb and Actg1 3'UTR binding microRNAs
Three miRNA prediction databases were used to analyzed possible miRNAs that target either/both Actb and Actg1 3'UTRs. Consensus miRNAs that were predicted in all databases are listed in the right column.

Supplemental Figure Legends

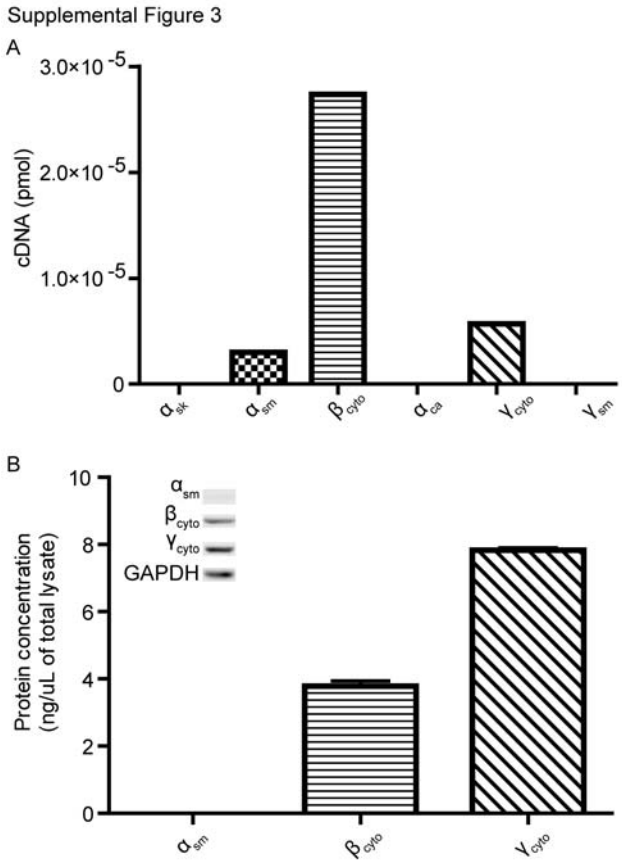


Supplemental figure 1: β_{cyto}-actin sKO SV40 LargeT antigen immortalized MEFs were not growth impaired**.** (A) Representative Western blot of SV40 LargeT antigen transformed (T) CT and KO MEF lysates blotted with α_{sm}-actin, β_{cyto}-actin and γ_{cyto}-actin antibodies; GAPDH served as loading control. (B-D) Growth curve analysis of SV40 LargeT antigen transformed (T) CT and KO MEFs (n=3, hand counted in duplicate). Asterisks denote *P<0.05, *** P<0.001. (Two-way ANOVA with Bonferoni post-test, error bars are s.e.m.).



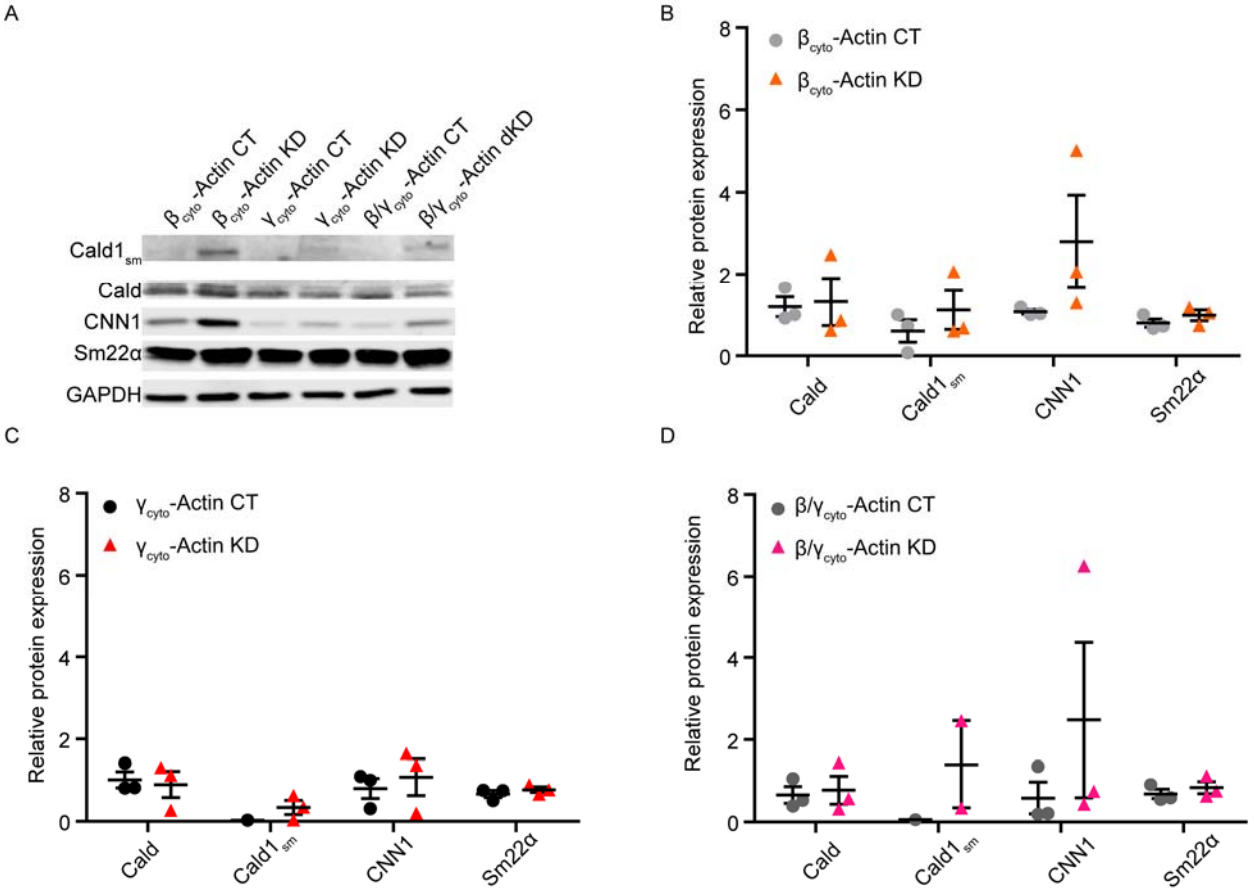
Supplemental figure 2: Mouse actin isoform standard curves and primer specificity analysis. (A-F) Representative standard curves generated using specific actin isoform primers with the corresponding control actin construct in a ten-fold dilution. (G) Representative graph of qRTPCR primer specificity. Each primer set was used to amplify all actin isoform control

constructs to calculate relative quantity. Color bars represent individual actin isoform, y-axis denotes relative quantity, x-axis denotes primer set.



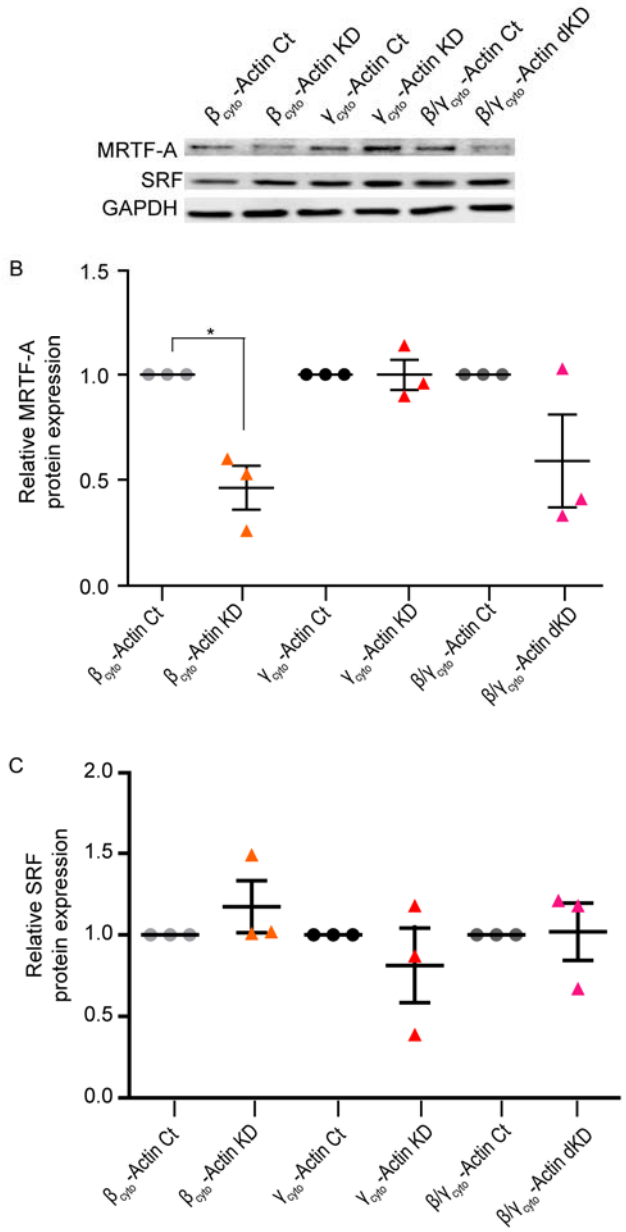
Supplemental figure 3: β_{cyto}- and γ_{cyto}-actin are the dominant actin isoforms in NIH3T3 fibroblast. (A) Actin isoform transcript profiling in NIH3T3 fibroblasts. Calculated transcript amount (pmol) were calculated based on the standard curve, amplified in parallel (n=1, in triplicate). (B) Representative Western blot of NIH3T3 fibroblast lysates blotted with α_{sm}-actin, β_{cyto}-actin and γ_{cyto}-actin antibodies; GAPDH served as loading control. Calculated protein concentrations (ng/μL of lysate) were determined based on the standard curve, blotted in parallel (n=1, in duplicate).

Supplemental Figure 4

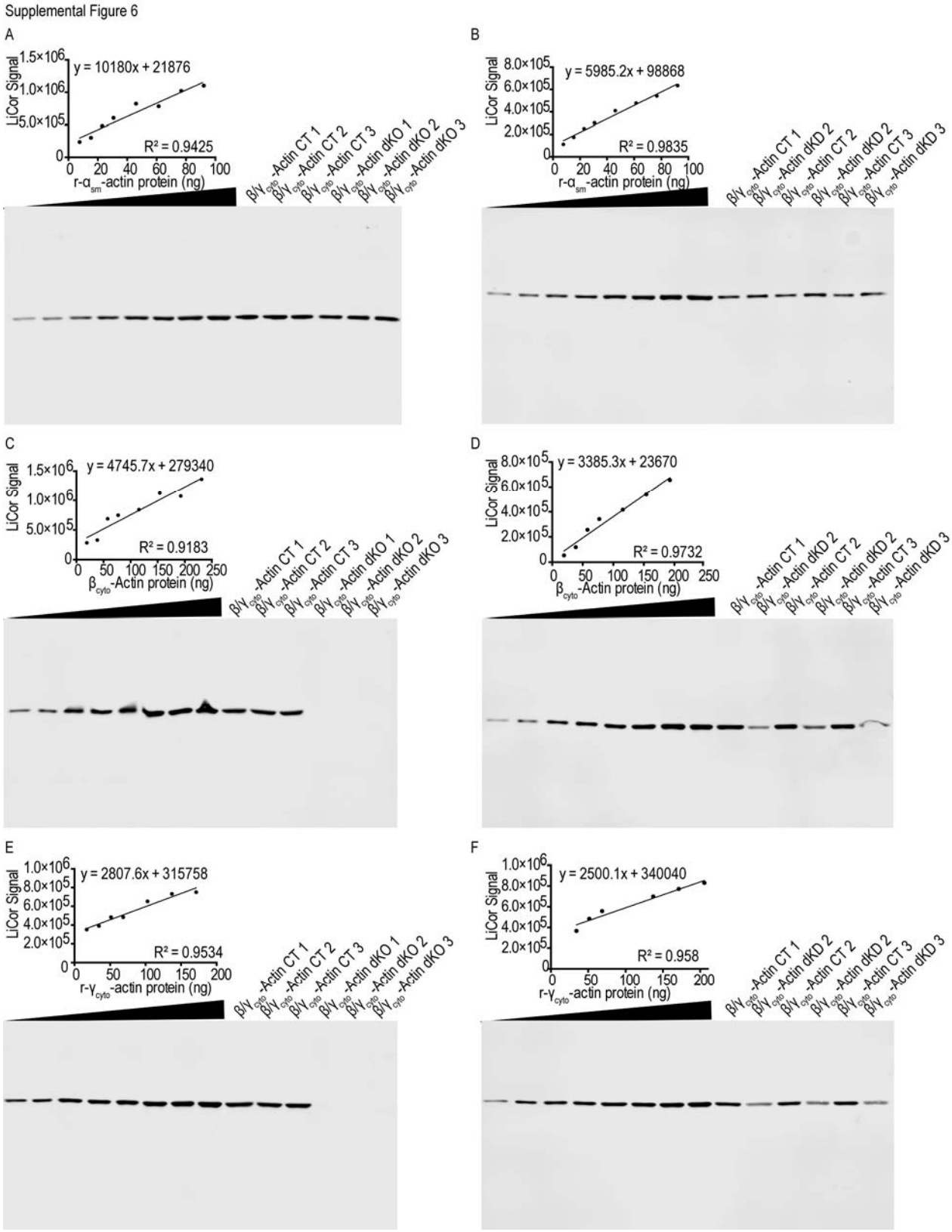


Supplemental figure 4: Cald1 and CNN1 protein expression are upregulated in β_{cyto}-actin siRNA KD MEFs. (A) Representative Western blot analysis of CT and KD MEFs at 3dpi for sKD and 4dpi for dKD blotted with Cald, CNN1, and Sm22α; GAPDH was used as loading control. (B-D) Relative protein expression were normalized to GAPDH and relative to the paired embryo control (n=3). (One sample T-test, error bars are s.e.m.).

Supplemental Figure 5



Supplemental figure 5: MRTF-A protein expression is down regulated ion β_{cyto}-actin KD MEFs. (A) Representative Western blot analysis of CT and KD MEFs at 3dpi for sKD and 4dpi for dKD blotted with MRTF-A and SRF; GAPDH served as loading control. (B) Calculated relative protein expression were normalized to GAPDH and relative to the paired embryo control (n=3). Asterisk denotes *P<0.05 (One sample T-test, error bars are s.e.m.).



Supplemental figure 6: Representative quantitative Western blots. (A, C, E) β/γ_{cyto}-Actin CT - and β/γ_{cyto}-Actin dKO, at 9dpi, from 3 separate embryos are blotted with α_{sm}-, β_{cyto}- and γ_{cyto}-actin antibodies. (B, D, F) β/γ_{cyto}-Actin CT - and β/γ_{cyto}-Actin dKD, at 4dpi, from 3 separate embryos blotted with α_{sm}-, β_{cyto}- and γ_{cyto}-actin antibodies. For α_{sm}-actin Western blots, both

KO and KD samples were diluted by at least 5-fold in order for the immune activity intensity to fit within the standard curve. An increasing amount of purified α_{sm}-, β_{cyto}- and γ_{cyto}-actins are blotted in parallel to generate a standard curve and used to calculate the amount of individual actin isoform in lysates.