Supplemental Information

Experimental Procedures S1.

Animals- Pregnant Sprague-Dawley (SD) rats and C57BL/6J mice were purchased from Sankyo Labo Service Corporation, Inc. All experiments were carried out in accordance with the guidelines of the Animal Care and Experimentation Committee of the University of Toyama, Sugitani Campus. All efforts were made to minimize the number of animals used and to minimize suffering.
Reagents- Activin and follistatin were prepared from bovine follicular fluid as described previously (1). Actinomycin D was purchased from Sigma and cycloheximide was from Wako Pure Chemicals.
Plasmids and Antibodies- The myc-tagged mouse MKL2 construct (Myc-MKL2) was generated as follows. Plasmid mKIAA1243 (carrying the MKL2 gene), provided by the KAZUSA DNA Institute (Chiba, Japan), was used as a template for site-directed mutagenesis with primer pairs (sense: 5'-GCAGGCTGTCGAATGAGATCTCATCGATAGCTCCAAG-3'; antisense: 5'-CTTGGAGCTATCGATGAGATCTCATTCGACAGCCTGC-3') to create a *Bgl*II site upstream of the initiation codon. The expression vector, pCMV-Myc (Clontech, USA), was mutated with primer pairs (sense: 5'-GCCATGGAGGCCGCGAATTCGGTCGAC-3'; antisense: 5'-GTCGACCGAATTCGCGGCCTCCATGGC-3') to create a *Bgl*II site downstream of the myc tag. A *Bgl*II/*Not*I-digested fragment including the MKL2 coding region was then ligated into the *Bgl*II/*Not*I site of the mutated pCMV-Myc. A FLAG-tagged full-length mouse MKL1 construct (FLAG-MKL1FL) identical to FLAG-MAL was used, and had been generated as previously reported (2). The FLAG-tagged mouse MKL1met (FLAG-MKL1met), which initiates the first methionine at the 93rd residue of the full-length mouse MKL1, was generated as follows. The FLAG-MKL1FL was mutated with primer pairs (sense: 5'-GAGCCAAGGGATGCGGCCGCCTTTGAAAAGCCC-3'; antisense: 5'-GGGCTTTTCAAAGGCGGCCGCATCCCTTGGCTC-3') to create a *Not*I site. The mutated construct was then self-ligated after *Not*I-digestion and a further mutation was created with primers (5'-GATGACGACAAGCTGCGGCCGCGAATTC-3' and 5'-GAATTCGCGGCCGCAGCTTGTCGTCATC-3') to remove the misssense mutation. The reporter vectors, pSRE-Luc (SRE-Luc) and pCRE-Luc (CRE-Luc) were purchased from Stratagene (La Jolla, CA, USA). An internal control vector, the RSV-βgal vector, has been described previously (3). The dominant negative SRF mutant, devoid of the C-terminal activation domain, SRFΔ, was a gift from Dr. S. Impey (Oregon Health & Science University, Portland, OR, USA). The myc-tagged SCAI plasmid was from Drs. D. T. Brandt and R. Grosse (University of Heidelberg, Germany). The dominant negative MKL1 mutants, ΔB1B2 and C471Δ were obtained from Dr. R. Treisman (Cancer Research Institute, London, UK). The expression vector for C3 transferase was obtained from Dr. A.Ghosh (UCSD, La Jolla, CA, USA). The reporter vector carrying the rat β-actin promoter (β-actin-Luc) was generated by the insertion of the β-actin gene promoter region (NCBI Reference Sequence: NW_047369.2; corresponding to nucleotides -489- +48 of rat β-actin) into the *Kpn*I/*Sac*I site of the pGL4.12 vector (Promega). The following antibodies were used at the indicated dilutions: Alexa Fluor® 448 or 594-conjugated anti-rabbit IgG (Invitrogen; 1:1000), Alexa Fluor® 448 or 594-conjugated anti-mouse IgG (Invitrogen; 1:1000), anti-MKL1 (C-19, sc-21558, Santa Cruz; 1:1000) and anti-MKL2 (C-19, sc-47282, Santa Cruz; 1:1000) made in goat, horseradish peroxidase (HRP)-conjugated anti-rabbit IgG, HRP-conjugated anti-mouse IgG (GE Healthcare; 1:5000) and HRP-conjugated anti-goat IgG (Santa Cruz) made in donkey. The following rabbit polyclonal antibodies were used: anti-green fluorescent protein (GFP) (Invitrogen or Medical & Biological Laboratories; 1:500), anti-SCAI (ECM Biosciences; 1:1000) and anti-RNA polymerase II (PolII) (Santa Cruz; 1:200). The following mouse monoclonal antibodies were used: anti-FLAG (SIGMA; 1:1000), anti-myc (Invitrogen; 1:1000, for immunostaining, Clontech; 1:500 for western blotting),

anti-microtubule associated protein 2 (MAP2) (Sigma ; 1:1000), anti-α-tubulin (Sigma; 1:1000) and anti-β-actin (Sigma; 1:1000).

Cell culture- Cells were grown in Dulbecco's modified Eagle medium (DMEM; Invitrogen) containing 10 % fetal bovine serum (FBS) (Invitrogen), 10 % Nu-serum (BD Biosciences, San Jose, CA, USA), 2 mM glutamine (Invitrogen) and 1 % penicillin/streptomycin (Invitrogen). For the reporter assays, cells were seeded at 5 x 10^5 cells/well and grown on six-well plates (Nalge Nunc). Dissociated cortical cell cultures were prepared from rat embryos at embryonic day 17 as described previously (4). A modified protocol was used and is described below. Cortical tissue was dissected quickly in DMEM (Invitrogen) containing 10 % FBS and 1 % penicillin/streptomycin (Invitrogen). After the dissected tissue was treated with trypsin solution and DNase I solutions, cells were re-suspended in neurobasal medium containing 1 x B27 supplement (Invitrogen), 2 μg/mL gentamicin, and 0.5 mM glutamine. Half of the conditioned medium was exchanged for fresh medium every three days. For immunostaining, cells were plated at a density of approximately 7 x 10^5 cells/well onto 18-mm circle coverslips coated with poly D-lysine that had been placed in 12-well plates. For reporter assays, cells were plated at a density of 2 x 10^6 cells per well in 6-well plates.

RNA isolation and PCR conditions for quantitative real-time PCR- Total RNA was extracted from mouse (C57BL/6J) tissues and brain regions using ISOGEN (Nippon Gene, Toyama, Japan) and cDNA was synthesized with SuperScriptII (Invitrogen). In brief, for detection of MKL1 and MKL2 mRNA levels, the PCR was performed in 25 μL of 1 x SYBR system using the SYBR Green PCR master mix (Applied Biosystems) containing 1 μL of cDNA solution and 0.5 μM primers (5'-TGGAGAGACGCTTTTTCTGG-3' and 5'-TGAGCTTCTTCACCTTTGGC-3' for MKL1; 5'-GAATGCCTTGAGGGAAGCAA-3' and 5'-AAGAGATGGCTCTGCAAACG-3' for MKL2). After preheating at 95 °C for 10 min, the samples were denatured at 95 °C for 45 s, annealed at 55 °C for 45 s, and extended at 72 °C for 1 min for 45 cycles.

Preparation of protein extracts for western blotting- NIH3T3 cells and cortical neurons were harvested and the protein was extracted with 160 μL of lysis buffer containing 25 mM Hepes (pH 7.7), 0.3 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.1 % Triton X-100, 20 mM β-glycerophosphate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM sodium orthovanadate, 1 mM DTT and 1 mM PMSF. After centrifugation, the cell lysates were mixed with an equal volume of 2 x Laemmli sample buffer (Bio-Rad Laboratories) and subjected to SDS-PAGE. To prepare protein extracts from mouse hippocampus, cortex and cerebellum, we homogenized 100 mg of frozen tissue in 250 μL lysis buffer. After centrifugation, the samples were processed as described above for culture lysates.

Plasmid construction and the assessment for RNA interference- To knock-down MKL2 expression, a vector expressing small interfering RNA (siRNA) (pSUPER-mrMKL2: siMKL2) was constructed. The following oligonucleotides (60-mers) (5'-GATCCCCGCCATCCCAAGAATCCAAATTCAAGAGATTTGGATTCTTGGGATGGCTTTTTA-3' and 5'-AGCTTAAAAAGCCATCCCAAGAATCCAAATCTCTTGAATTTGGATTCTTGGGATGGCGGG-3') were annealed and subcloned into *Hind*III/*Bgl*II sites of the pSUPER vector (OligoEngine Platform, Seattle, WA, USA). The target sequences are identical to both mouse and rat MKL2 genes. The control vector, pSUPER-mrMKL2mut (siMKL2m), which has two base substitutions, was also generated by the annealing and subcloning of the oligonucleotides (5'-GATCCCCGCCATCCCTAGAAGCCAAATTCAAGAGATTTGGCTTCTAGGGATGGCTTTTTA-3' and 5'-AGCTTAAAAAGCCATCCCTAGAAGCCAAATCTCTTGAATTTGGCTTCTAGGGATGGCGGG-3'). For MKL1 knock-down, pSUPER-mrMAL (siMKL1) and its control vector, pSUPER-mrMALm (siMKL1m), reported previously (2), were used. Transfection of these pSUPER vectors was performed and the effects were assessed by western blotting (Fig. 7 and supplemental Fig. S4).

1. Nakamura, T., Asashima, M., Eto, Y, Takio, K., Uchiyama, H., Moriya, N., Ariizumi, T., Yashiro, T., Sugino, K., Titani, K. and Sugino, H. (1992) *J. Biol. Chem.* **267**, 16385-16389.
2. Shiota, J., Ishikawa, M., Sakagami, H., Tsuda, M., Baraban, J. M. and Tabuchi, A. (2006) *J. Neurochem.* **98**, 1778-1788.
3. Tabuchi, A., Sakaya, H., Kisukeda, T, Fushiki, H. and Tsuda, M. (2002) *J. Biol. Chem.* **277**, 35920-35931.
4. Tabuchi, A., Nakaoka, R., Amano, K., Yukimine, M., Andoh, T., Kuraishi, Y. and Tsuda, M. (2000) *J. Biol. Chem.* **275**, 17269-17275.

Supplemental Figure legends

Figure S1. Double staining of cortical neurons with anti-GFP and anti-MAP2 antibodies. (A and B) GFP-expressing cells, subjected to the morphological analyses performed in Figs. 2 and 3, respectively, were MAP-2 positive neurons.

Figure S2. The effect of activin on CRE- and SRE-reporter activation. The reporter vectors of CRE-Luc (A) or SRE-Luc (B) were transfected into cortical neurons. Twenty-four hours later, the cells were stimulated with activin (100 ng/ml) for 24 h. Bar graphs represent the mean ± S.D. from three samples. The same trends were obtained from at least two independent experiments. $^{**}p<0.01$; $^{***}p<0.001$. (C) Time-course of SRF-mediated transcriptional response induced by activin. The transfection procedure was the same as (A). Transcriptional activity was measured at the times indicated after activin-stimulation. Bar graphs represent the mean ± S.D. from four samples. $^{*}p<0.05$.

Figure S3. Time-course of dendritic complexity induced by activin. Sholl analysis was performed at the times indicated (A: 0 h; B: 0.5 h; C: 1 h; D: 3 h; E: 6 h; F: 12 h; G: 24 h; H: 48 h) after activin-stimulation (100 ng/ml). Graphs represent the mean ± S.D. from four independent experiments. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

Figure S4. Specific knock-down of MKL1 and MKL2 expression by their own target siRNAs. FLAG-MKL1FL (A) or myc-MKL2 (B) (0.8 μg/well) was co-transfected with GFP (0.4 μg/well) and either pSUPER (empty, 0.8 μg/well) or siMKL2 (0.8 μg/well) or siMKL1 (0.8 μg/well) into NIH3T3 cells. Seventy-two hours later, the samples were subjected to western blotting with anti-FLAG (panel A, upper), anti-myc (panel B, upper), anti-α-tubulin for constant sample applications (middle) or anti-GFP antibodies for constant transfection efficiencies (lower). (A) The expression of FLAG-MKL1FL displays a clear reduction with siMKL1, but not with siMKL2. (B) The expression of myc-MKL2 displays a clear reduction with siMKL2, but not with siMKL1.

Figure S5. MKL1 siRNA reduces activin-induced SRF transcriptional activation and dendritic complexity. The experimental procedures were the same as presented in Fig. 6, except siMKL1was used instead of siMKL2. (A) Effect of MKL1 siRNA on SRE-reporter activity. Reporter vectors (1 μg/well) were cotransfected with empty (pSUPER) or siMKL1 or siMKL1m (3 μg/well) into cortical neurons and activin was added at 48 h post-transfection. Bar graph represents the mean ± S.D. from four samples. The same trends were obtained from at least two independent experiments. $^{**}p<0.01$; $\#p<0.05$; $\#\#\#p<0.001$; N.S. $p>0.05$. (B) MKL1 siRNA reduces dendritic complexity. Empty (pSUPER), siMKL1 or siMKL1m (2 μg/well) was co-transfected with a GFP construct (2 μg/well) and the neurons were stimulated with activin (Act+) 48 h post-transfection. Another 24 h later, cells were fixed and subjected to immunostaining. (C and D) Dendritic complexity analyzed in the experimental conditions shown in (B). Graphs represent the mean ± S.D. from five independent experiments. (C) Sholl analysis. The supplemental Table 1 shows the statistical significance. (D) Dendritic length. $^{*}p<0.05$; $\#p<0.05$; N.S. $p>0.05$.

Figure S6. Dominant negative MKL1 mutants suppress the increase of SRF-mediated transcription induced by activin. (A) Structure of wild-type (on the top) and dominant negative MKL1. The mutant termed ΔB1B2 lacks the nuclear import region in response to Rho signaling whereas the C471Δ mutant lack the transcription activation domain. (B) Cortical neurons were co-transfected with reporter vectors and either the empty vector or ΔB1B2 or C471Δ. Twenty-four hours later, cells were stimulated with

activin (100 ng/ml) for 24 h and the samples were assayed. Bar graph represents the mean ± S.D. from three samples. The same trends were obtained from at least two independent experiments. *p<0.05; **p<0.01; ##p<0.01; ###p<0.001.

Figure S7. Endogenous MKL1 and MKL2 proteins, localized in the nucleus and cytoplasm of cortical neurons stimulated with activin. (A and B) Specific detection of MKL1 and MKL2 by anti-MKL1 and anti-MKL2 antibodies, respectively. The strong bands probed by the anti-MKL1 antibody were obtained from the lysate of NIH3T3 cells transfected with FLAG-MKL1FL (lane 2) or FLAG-MKL1met (lane 3), but not from the cells transfected with the empty vector for MKL1 (lane 1), empty vector for MKL2 (lane 4) or myc-MKL2 (lane 5). (B) Conversely, the strong bands probed by anti-MKL2 antibody were obtained from the lysate of NIH3T3 cells transfected with myc-MKL2 (lane 2), but not from the cells transfected with the empty vector for MKL2 (lane 1), empty vector for MKL1 (lane 3), FLAG-MKL1FL (lane 4) or FLAG-MKL1met (lane 5). (C-J) Detection of MKL1 and MKL2 with nuclear and cytoplasmic extracts of activin-stimulated cortical neurons. Cortical neurons after 9 days of *in vitro* culturing were stimulated with activin (100 ng/ml) for the times indicated. Subsequently, nuclear and cytoplasmic extracts were prepared and subjected to western blotting. Western blotting with nuclear extracts (C, D, G and H) or with cytoplasmic extracts (E, F, I, and J). Detection of the endogenous MKL1 protein with the anti-MKL1 antibody (C and E) or that of the endogenous MKL2 protein with the anti-MKL2 antibody (D and F). Separation of nuclear and cytoplasmic extracts was confirmed with antibodies against RNA polymerase II (PolII) (G and I) and α-tubulin (H and J), which are nuclear and cytoplasmic markers, respectively.

Figure S8. Inhibition of activin- or MKL2-induced SRF transcriptional activation and dendritic complexity by C3 transferase. (A-D) Activin (100 ng/ml) was added 24 h post-transfection. (E-H) The MKL2 vector was cotransfected with the vectors described below. (A and E) An empty vector (empty) or the expression vector for C3 transferase (C3, 0.1 μg/well) was co-transfected with the reporter vectors in cortical neurons. Bar graphs represent the mean ± S.D. from four samples. The same trends were obtained from at least two independent experiments. **p<0.01; ***p<0.001; ###p<0.001. (B and F) Attenuation of dendritic complexity by C3 transferase. Immunofluoresent images of cortical neurons expressing GFP (2 μg/well) and either an empty vector (empty, 2 μg/well) or C3 transferase (C3, 0.1 μg/well). (C, D, G, and H) Dendritic complexity analyzed in the experimental conditions shown in (B and F). Graphs represent the mean ± S.D. from four independent experiments. (C and G) Sholl analysis. The supplemental Table 1 shows the statistical significance. (D and H) Dendritic length. **p<0.01; ***p<0.001; ### p<0.001.

Figure S9. MKL mediates SRF-target β-actin gene transcription. The expression vector for either FLAG-MKL1FL (MKL1FL) (A) or myc-MKL2 (MKL2) (B) (3 μg/well) was co-transfected with β-actin-Luc, a reporter vector carrying the rat β-actin gene promoter (1 μg/well) in cortical neurons. Luciferase activity was measured 48 h post-transfection. (C) MKL knock-down impaired activin-induced β-actin gene promoter activation. Reporter vectors (1 μg/well) were cotransfected with an empty or siMKL1, siMKL1m, siMKL2, siMKL2m (3 μg/well) vectors into cortical neurons and activin was added 48 h after transfection. Bar graphs represent the mean ± S.D. from at least four samples. The same trends were obtained from at least two independent experiments. **p<0.01; ***p<0.001; ###p<0.001; N.S. p>0.05.

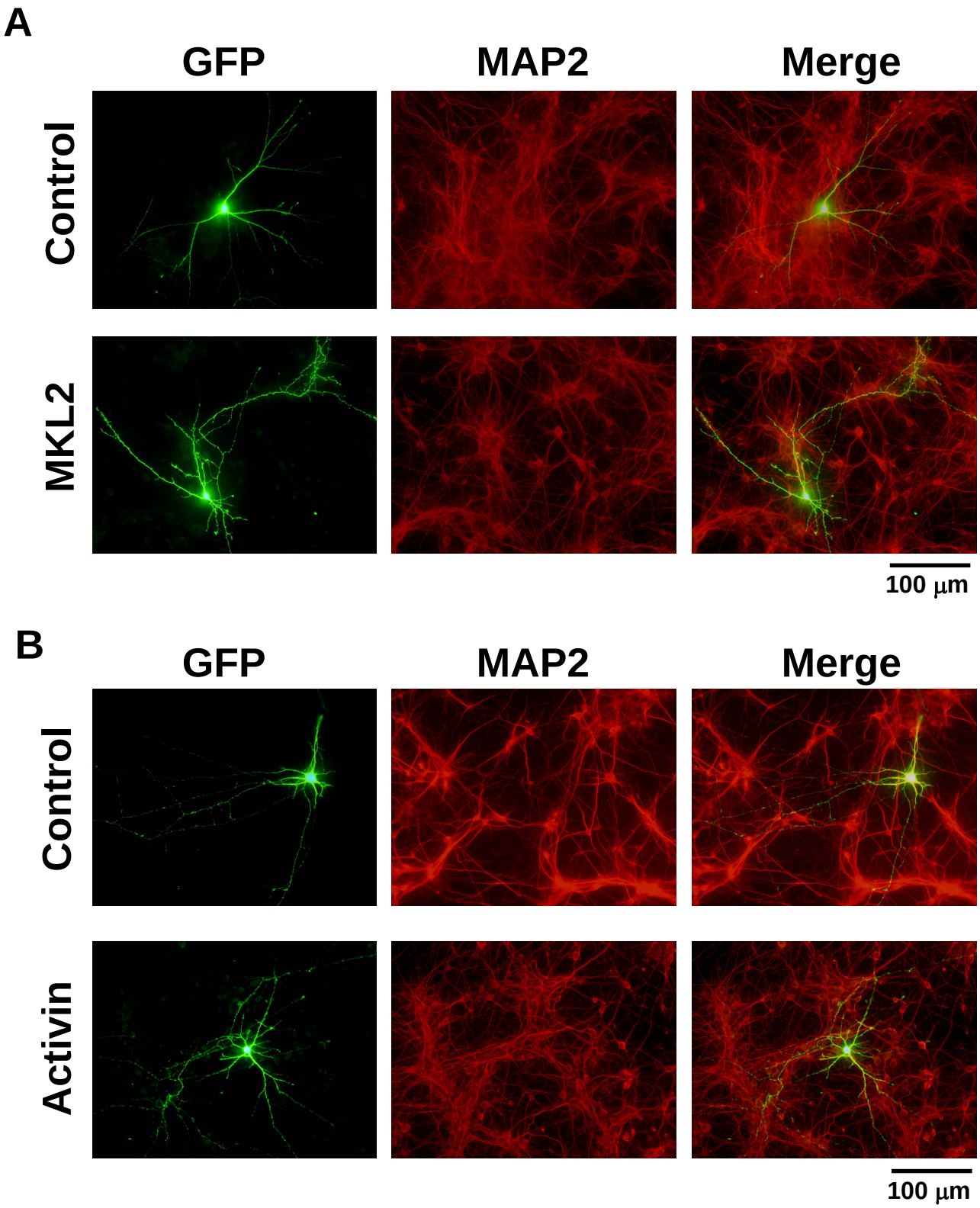
Supplemental Table 1

Statistical significance of sholl analysis through this study

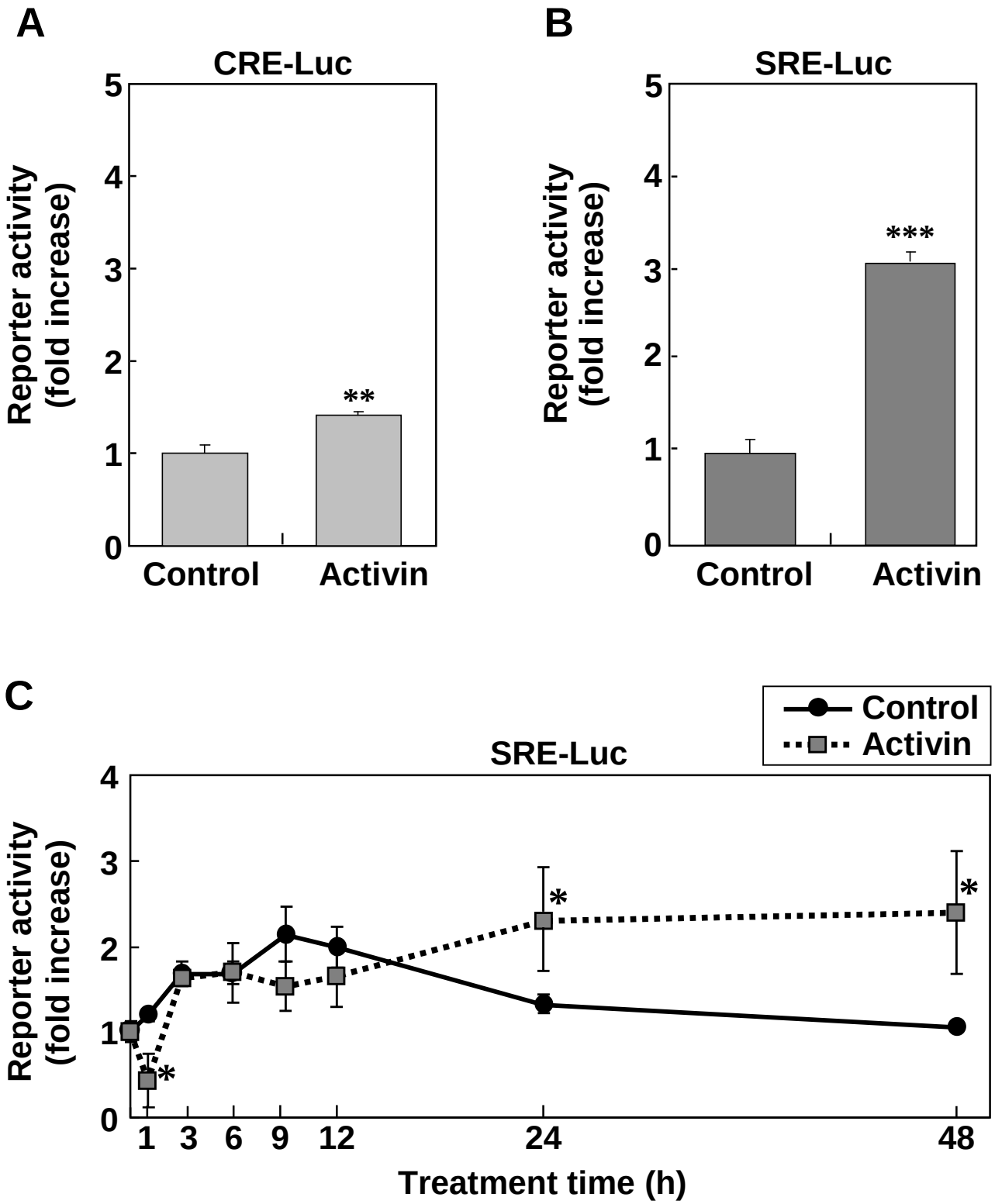
			Distance from the cell body (μm)									
			20	40	60	80	100	120	140	160	180	200
Figure 3C	Control	FLS -	5.73±0.30	7.55±0.78	8.03±0.37	7.18±1.30	6.83±1.10	4.98±0.92	4.70±0.31	3.70±0.48	3.13±0.76	1.95±0.37
		FLS +	5.15±0.75	5.88±0.01	7.03±0.53	7.43±1.42	5.65±1.04	4.45±0.37	3.50±0.55	3.15±0.35	2.45±0.47	1.45±0.26
	Activin	FLS -	7.45±0.44	8.75±0.72	8.75±0.79	9.25±0.60	7.80±0.85	6.80±0.47	5.98±0.10	4.60±0.75	3.80±0.76	3.05±0.65
		FLS +	6.28±0.87	7.75±0.75	6.95±0.82	6.95±0.45	6.00±0.66	4.73±0.59	4.10±0.70	4.03±1.01	2.73±0.71	1.88±0.53
Figure 4B	Control	ACTD -	5.13±0.46	6.30±0.59	6.93±0.83	7.23±0.54	6.73±0.67	4.85±0.53	4.13±0.60	3.33±0.40	2.08±0.53	1.70±0.44
		ACTD +	3.70±0.18	4.88±0.45	3.80±0.36	3.10±0.57	2.70±0.54	2.10±0.57	1.43±0.31	1.28±0.39	0.83±0.31	0.55±0.42
	Activin	ACTD -	7.15±0.94	9.00±0.94	8.83±0.53	8.33±0.56	7.80±0.52	6.83±0.68	5.53±0.64	4.03±0.59	3.30±0.72	2.80±0.35
		ACTD +	3.98±0.82	5.45±0.77	4.98±0.63	4.75±0.58	2.63±0.73	1.83±0.66	1.58±0.48	1.53±0.60	0.98±0.40	0.78±0.33
Figure 4E	Control	CHX -	5.45±0.47	7.28±0.67	7.30±0.75	7.83±0.44	6.43±0.86	4.85±0.53	4.55±0.66	3.58±0.46	2.45±0.25	1.98±0.35
		CHX +	3.23±0.47	3.98±0.88	5.23±0.95	4.83±1.15	3.50±0.58	2.63±0.40	1.70±0.42	1.43±0.38	0.78±0.33	0.08±0.10
	Activin	CHX -	7.08±0.47	8.90±0.94	9.23±0.62	8.68±0.85	7.50±0.52	6.93±0.53	5.95±0.71	4.48±0.60	3.55±0.76	2.83±0.71
		CHX +	3.35±0.44	4.10±0.37	3.88±0.84	3.85±0.51	3.40±0.78	2.13±0.76	2.00±0.86	1.93±1.20	1.38±0.84	0.53±0.30
Figure 5C	Control	empty	4.87±0.50	7.50±0.69	7.50±0.40	8.10±1.11	7.23±0.67	6.27±0.78	3.63±0.35	3.10±0.26	1.23±0.21	1.03±0.21
		SRFΔ	3.23±0.49	4.40±0.98	4.90±0.95	4.27±0.85	3.97±0.86	2.87±0.95	2.17±0.85	1.83±0.21	0.70±0.10	0.13±0.06
	Activin	empty	7.43±1.72	9.00±0.85	9.83±0.31	9.10±1.11	8.60±1.21	7.03±0.87	6.37±0.40	5.13±0.68	2.60±0.95	1.87±0.51
		SRFΔ	4.33±0.49	4.90±1.00	7.27±1.59	6.57±1.69	5.37±1.42	3.27±0.15	2.80±0.26	1.90±0.10	1.53±0.55	0.73±0.15
Figure 6D	Control	empty	6.28±0.44	7.65±0.69	7.66±0.55	7.52±0.92	6.07±0.78	5.29±0.47	4.54±0.83	4.02±0.69	2.88±0.23	1.85±0.19
		siMKL2	5.00±0.45	5.51±1.16	5.32±1.36	4.01±0.84	4.31±0.43	3.51±0.01	2.00±0.47	1.58±0.54	1.12±0.51	0.73±0.04
		siMKL2m	6.90±0.26	6.32±0.75	7.00±0.25	6.54±1.15	5.98±0.74	5.56±0.22	3.10±0.90	1.54±0.74	1.31±0.96	1.21±0.16
	Activin	empty	6.68±0.29	8.78±1.10	9.13±1.31	8.75±1.14	7.75±0.55	7.00±0.57	6.90±0.55	5.95±0.47	5.30±0.49	4.10±0.41
		siMKL2	5.48±1.14	6.90±1.11	5.90±0.87	4.29±0.70	4.12±0.73	3.99±0.34	3.51±0.67	2.42±0.60	1.50±0.48	0.80±0.05
		siMKL2m	7.09±0.33	8.10±1.59	7.77±0.67	7.54±1.66	7.75±0.41	6.99±0.30	6.90±0.33	5.34±0.62	2.98±0.96	2.56±0.46
Figure 8C	Control	empty	5.80±1.09	6.80±1.50	7.15±1.32	7.18±1.01	6.53±0.98	5.55±0.73	4.03±0.69	3.65±0.44	2.88±0.57	1.78±0.64
		SCAI	4.03±0.58	5.43±0.59	5.43±1.13	3.93±1.34	2.93±1.37	2.68±1.07	1.78±0.67	1.43±0.40	0.93±0.47	0.80±0.48
	Activin	empty	7.88±0.88	8.95±0.85	9.33±1.11	8.33±1.05	8.15±0.78	7.38±0.80	6.53±1.07	4.83±0.39	3.03±0.90	2.43±0.74
		SCAI	5.75±0.91	6.20±0.59	6.18±0.71	5.93±0.67	4.25±0.78	2.83±0.99	1.93±0.81	1.85±0.79	1.65±0.61	1.03±0.57
Supplemental Figure S5C	Control	empty	6.00±0.45	7.43±0.76	7.63±0.63	7.55±0.84	6.35±0.70	5.78±0.76	4.80±0.88	4.63±0.81	3.78±0.81	2.48±0.62
		siMKL1	5.65±0.50	5.83±1.37	5.03±1.00	4.98±1.09	4.95±1.08	4.15±1.12	4.05±1.13	3.50±0.88	3.00±0.66	0.90±0.73
		siMKL1m	6.18±0.85	6.95±0.66	8.00±0.84	7.75±1.42	6.33±0.97	5.13±0.96	4.85±0.90	4.23±0.97	3.10±0.86	2.35±0.79
	Activin	empty	6.85±0.79	8.45±1.62	9.15±0.75	8.53±1.41	7.65±1.07	6.10±0.93	5.83±1.13	5.13±1.28	4.78±1.62	3.05±0.70
		siMKL1	5.40±0.97	6.28±1.54	5.40±1.47	5.23±1.28	4.83±1.02	4.85±1.18	4.68±1.04	3.90±0.73	2.08±0.34	1.03±0.22
		siMKL1m	6.98±0.77	8.23±1.75	8.25±1.84	8.10±1.94	6.80±1.95	6.45±1.77	6.00±1.50	4.88±1.42	4.50±1.17	1.75±0.86
Supplemental Figure S8C	Control	empty	4.93±0.41	7.03±0.59	7.28±0.48	7.70±0.49	7.00±0.90	5.30±0.80	4.60±0.37	3.90±0.38	2.20±0.42	2.35±0.48
		C3	3.03±0.58	3.95±0.83	3.88±0.51	4.08±0.74	3.65±0.66	2.20±0.56	1.53±0.34	1.30±0.29	0.73±0.22	1.78±0.54
	Activin	empty	6.03±0.86	9.23±1.18	8.88±0.90	8.78±1.09	8.58±1.04	7.20±1.15	5.95±0.64	4.48±0.59	3.63±0.48	0.13±0.05
		C3	3.33±0.64	3.73±0.54	5.05±0.79	4.58±0.46	3.43±0.40	2.55±0.39	2.00±0.64	1.60±0.55	1.48±0.60	1.90±0.70
Supplemental Figure S8G	Control	empty	6.28±0.44	7.63±0.69	7.68±0.55	7.23±0.92	6.55±0.78	5.63±0.47	4.73±0.83	4.38±0.89	3.60±0.56	0.33±0.22
		C3	5.43±0.49	5.60±1.16	5.25±1.71	4.85±0.86	4.65±1.33	4.25±1.05	3.20±1.46	1.88±1.52	1.28±0.68	1.00±0.25
	MKL2	empty	6.95±0.29	9.08±1.10	9.30±1.31	8.63±1.26	7.75±0.77	6.70±0.69	6.48±0.41	5.83±0.99	5.03±1.43	3.63±0.88
		C3	5.33±1.18	5.58±1.11	5.38±1.25	4.90±1.10	4.95±0.94	4.65±0.90	3.98±1.57	2.95±1.08	1.95±0.47	1.20±0.18

*p<0.05; **p<0.01; ***p<0.001; #p<0.05; ##p<0.01; ###p<0.001

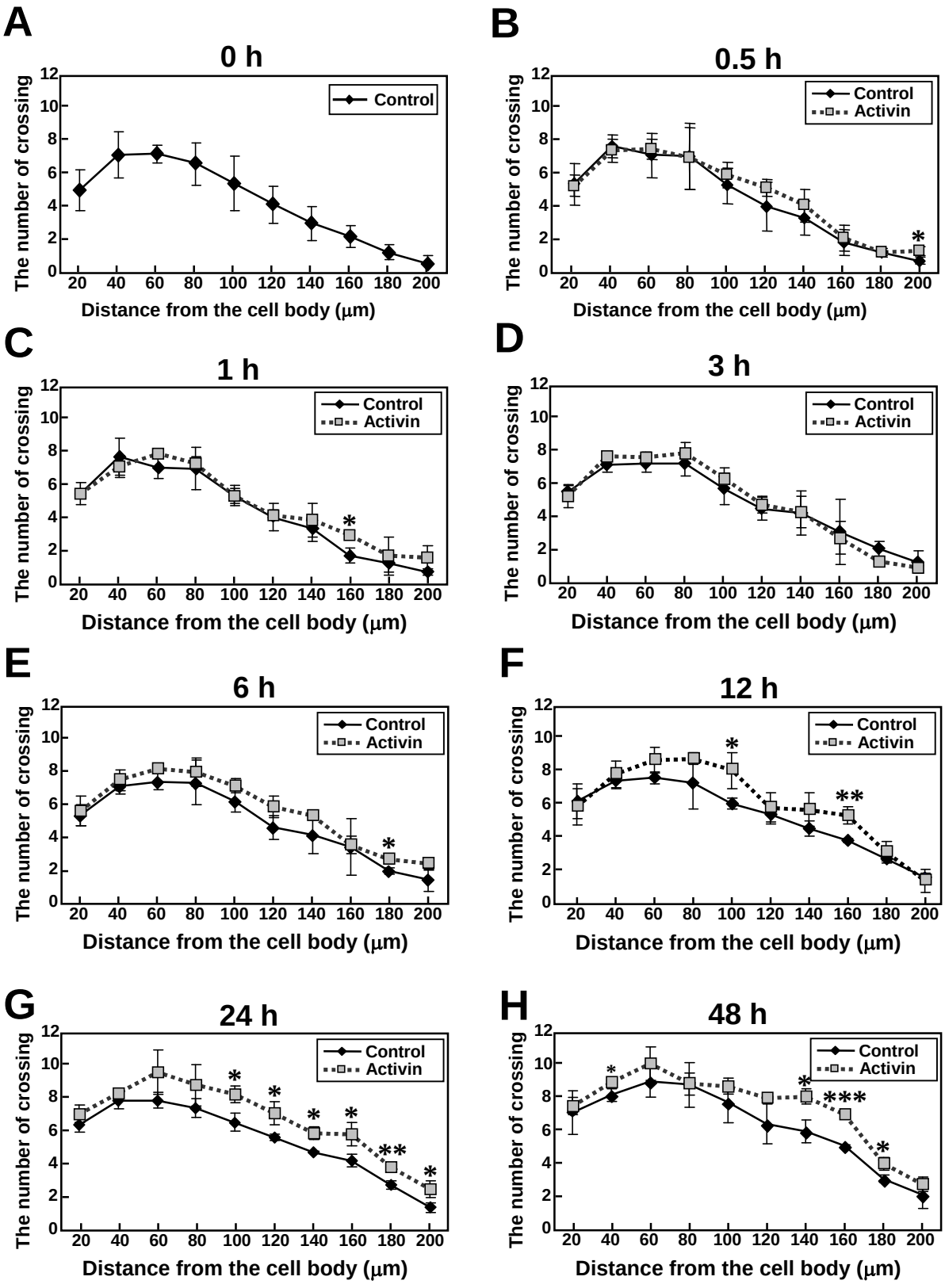
Supplemental Figure S1



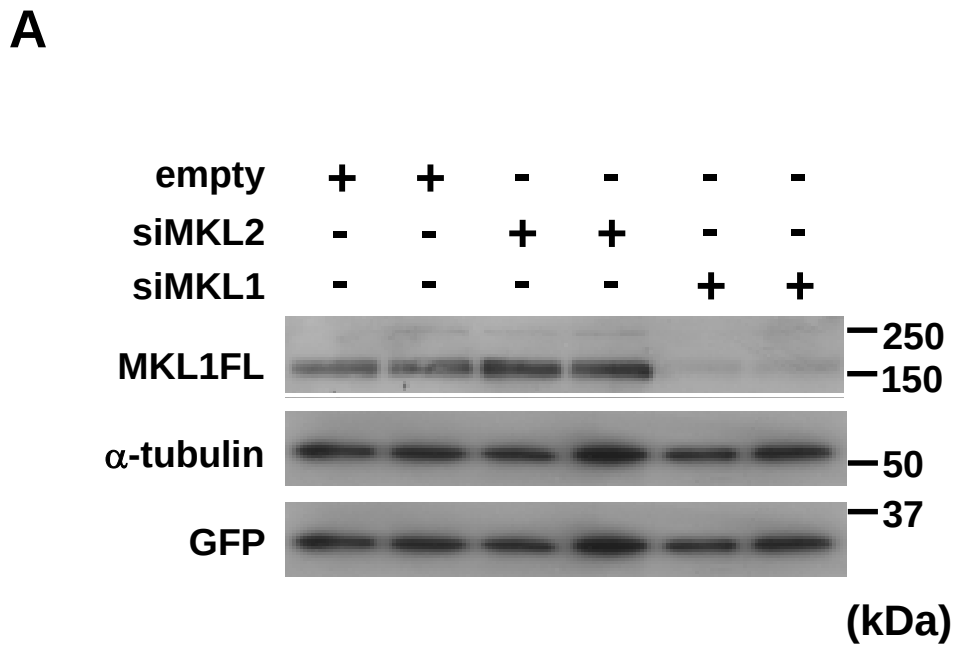
Supplemental Figure S2

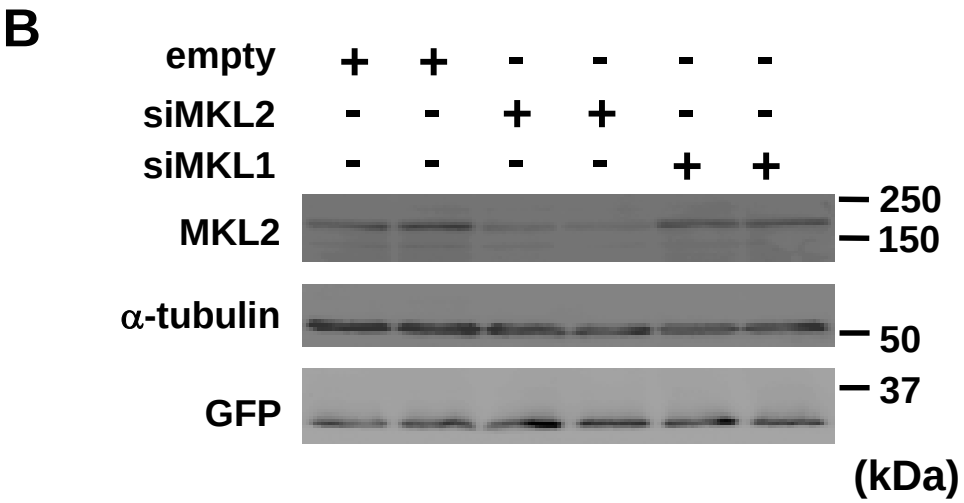


Supplemental Figure S3

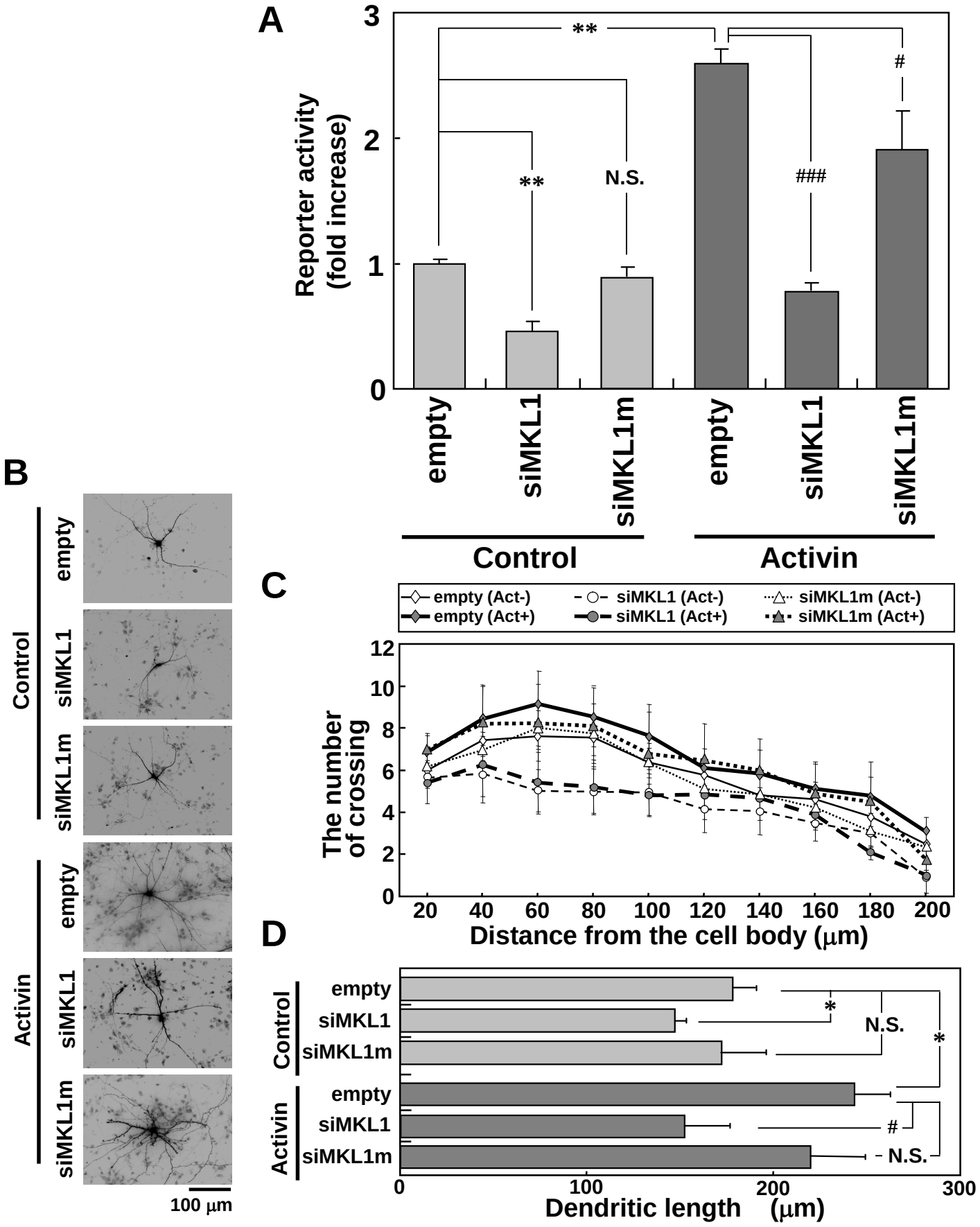


Supplemental Figure S4



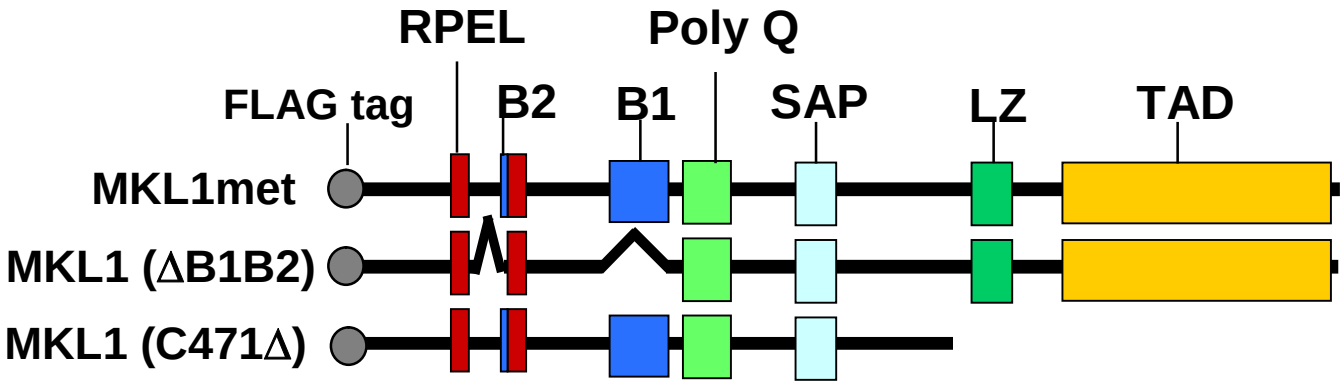


Supplemental Figure S5

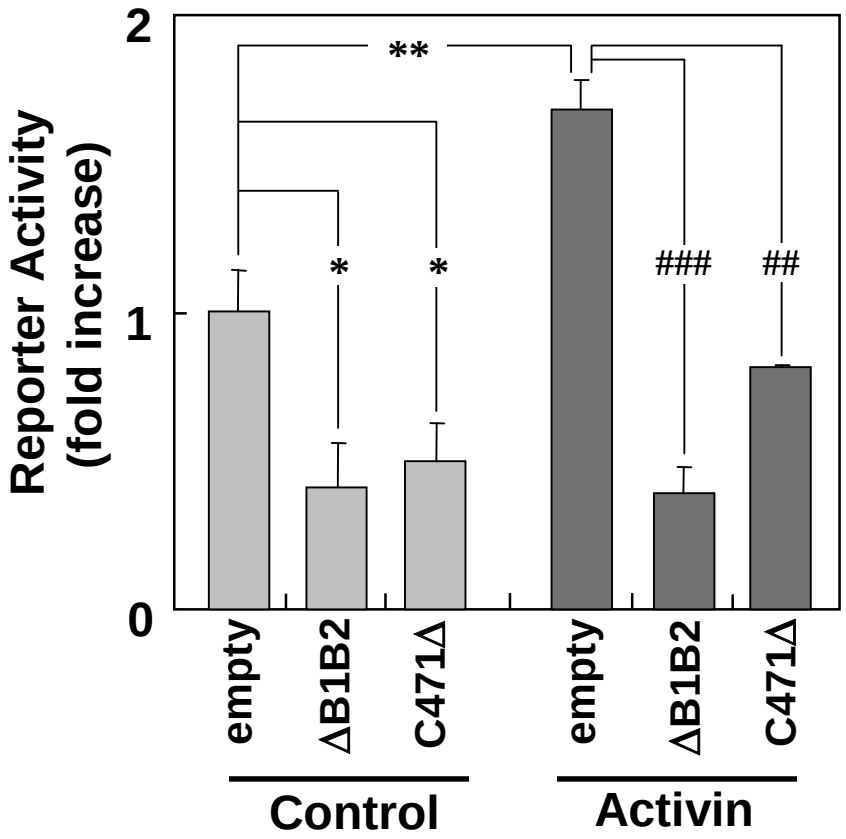


Supplemental Figure S6

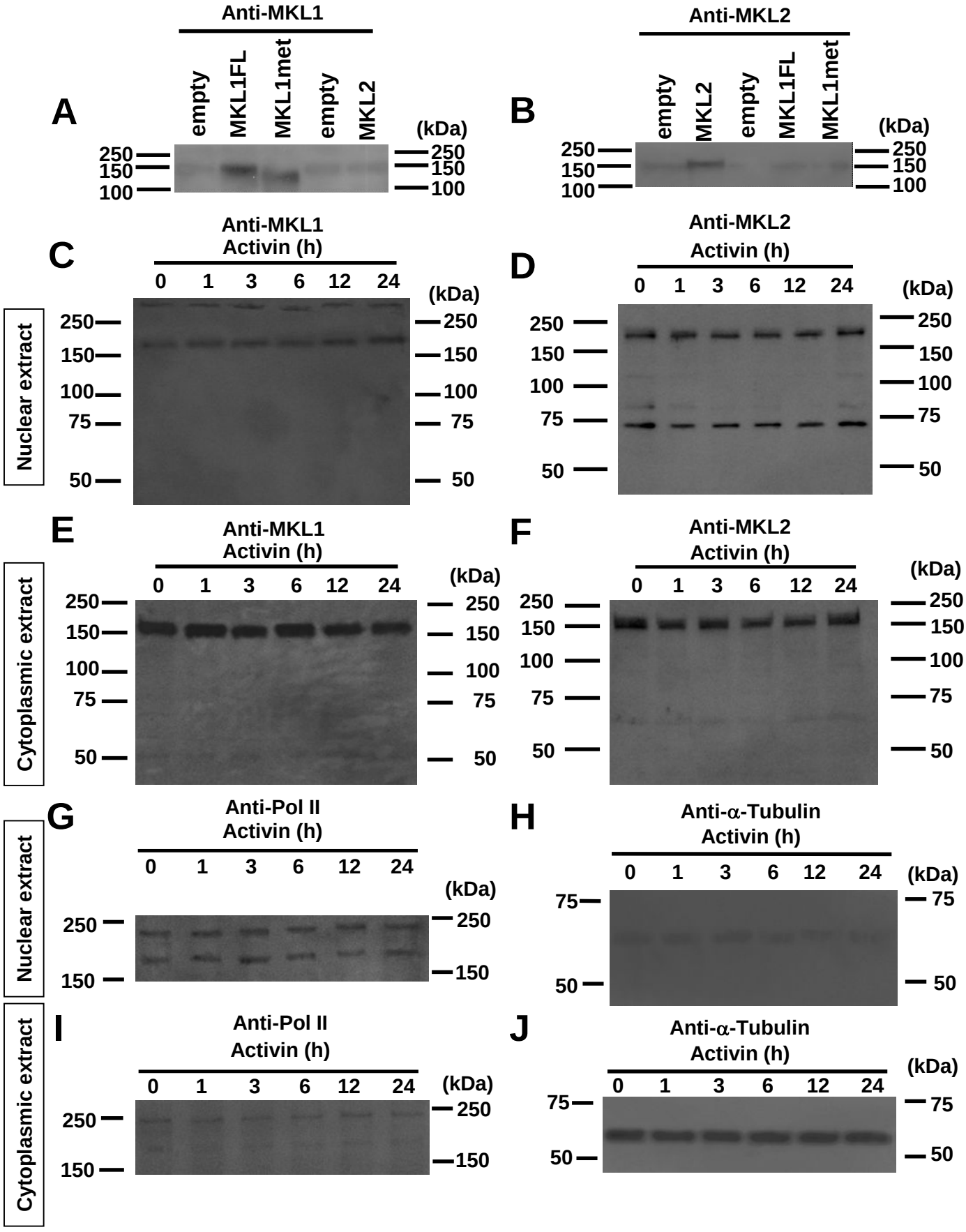
A



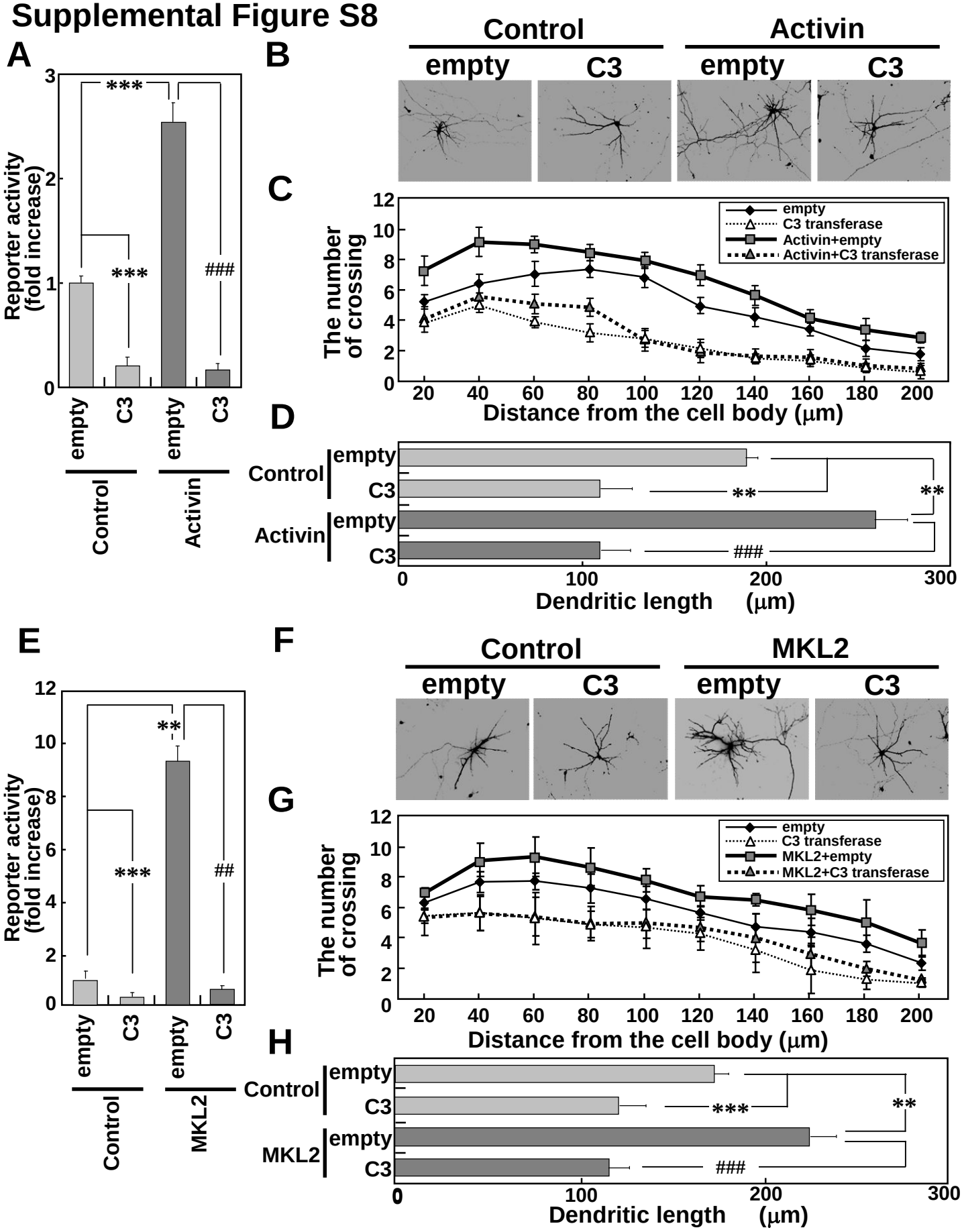
B



Supplemental Figure S7



Supplemental Figure S8



Supplemental Figure S9