Supplemental Table 1

Sequences, expected sizes, and GenBankTM accession numbers for primers used in RT-PCR and real time PCR

Gene	Forward primers (5'-3')	Reverse primer (5'-3')	Size (bp)	Accession no.
mBMP2	CTAGATCTGTACCGCAGGCACTC	CATCTCTGGAAGTTCCTCCACGG	135	NM_007553
mBMP4	GCCAACACTGTGAGGAGTTTCC	GATGCTGCTGAGGTTGAAGAG	102	NM_007554
mRGMb	GCTACACACTGGAGACTGCCA	AGTTGGCATCACCAGTGGTGAG	109	NM_178615
mJAM-A	ACGTCCAGGTTCCCGAGAAC	GTGCTGCCTTGGACGAACTTC	97	NM_172647
mJAM-B	ACT GAG CAA GGC CAG AAC CTG	CGT AGC TCC ACC ACA CTT CCA G	118	NM_023844
mJAM-C	GACTCACAGACAAGTGACCCTAGG	CCAAACACATCTGTGCGACCTG	119	BC024357
mZO-1	CACCAAGGTCACACTGGTGAAG	GAATGTCACCATCTCTTGCTGCC	119	NM_009386
mZO-2	CCATGGAAGATGTGCTCCATTCG	AACCTCGGTCGTCATGACTGAG	144	NM_011597
mZO-3	CGGTCAGTGAATTCAAGAGCCATC	GAAGCTCACGACACGAGTGTC	132	NM_013769

Supplemental Table 2

Sequences, expected sizes, and GenBankTM accession numbers for primers used in RT-PCR for IMCD3 cells

Gene	Forward primers (5'-3')	Reverse primer (5'-3')	Size (bp)	Accession no.
mBMP2	AGTTGAGGCTGCTCAGCATG	GGTGTCCAATAGTCTGGTCACAG	419	NM_007553
mBMP4	GCCGTCATTCCGGATTACATGAG	GTCGTGTGATGAGGTGTCCAG	370	NM_007554
mBMP5	ATGGAGAAGCAGTGACAGCAG	GCGTCTCTGCACAGAGCTGTAAG	260	NM_007555
mBMP6	GCTCTATGTGAGCTTCCAGGAC	GCTCTCACGACCATATTCCTGTAC	280	NM_007556
mBMP7	CGACATGGTCATGAGCTTCGTC	TTGATGCTCTGCCCATCCAG	384	NM_007557

Legends for supplemental figures

Supplemental Figure 1. Expression of Dragon mRNA and protein in the mouse kidney. (A) Northern blot of Dragon mRNA in mouse tissues. Total RNA was isolated from various adult mouse tissues and embryos (E15.5) using Trizol reagent and 10 µg of total RNA were used per tissue per lane. The sizes of mRNA are labeled as indicated. (B) Western blot analysis of whole kidney lysates using the anti-Dragon antibody in the presence (lane 1) or absence (lane 2) of competing immunizing-peptide.(C) Western blot analysis of HEK 293 cells lysates transfected with mock (lane 1) or Dragon cDNA (lane 2) using the anti-Dragon antibody.

Supplemental Figure 2. BMP2 and Dragon signals are additive. mIMCD3 cells were transfected with the BRE-Luciferase construct in the absence or presence of Dragon cDNA. Cells were then incubated without (open bars) or with BMP2 (filled bars) and relative luciferase activity was measured from cells extracts.

Supplemental Figure 3. Phospho-Smad1/5/8 is localized in glomeruli (A and B), collecting ducts (C-H), distal convoluted tubules/connecting segments (I-K) and thick ascending limbs (L-N) in the mouse kidney. (A and B) Nuclear staining of phospho-Smad1/5/8 in glomeruli. Mouse kidney sections were immunostained with rabbit anti-p-Smad1/5/8 antibody (A) or non-specific rabbit IgG (Control, B). Sections were then stained with Cy3-donkey anti-rabbit. G, glomerulus. (C-E) Nuclear staining of phospho-Smad1/5/8 in principal cells of collecting ducts. Mouse kidney sections were immunostained with rabbit anti-p-Smad1/5/8 antibody (C) and goat anti-aquaporin-2 (α-AQP2, D). Sections were then stained with Cy3-donkey anti-rabbit (red) and FITC-donkey anti-goat (green) secondary antibodies to visualize phospho-Smad1/5/8 and AQP2. Merged image is shown in panel E. (F-H) Nuclear staining of phospho-Smad1/5/8 in intercalated cells of collecting ducts. Mouse kidney sections were immunostained with rabbit anti-p-Smad1/5/8 antibody (F) and chicken anti-V-ATPase E1 antibody (G). Sections were then stained with Cy3-donkey anti-rabbit (red) and FITC-donkey anti-chicken (green) secondary antibodies to visualize phospho-Smad1/5/8 and V-ATPase. Merged image is shown in panel H.

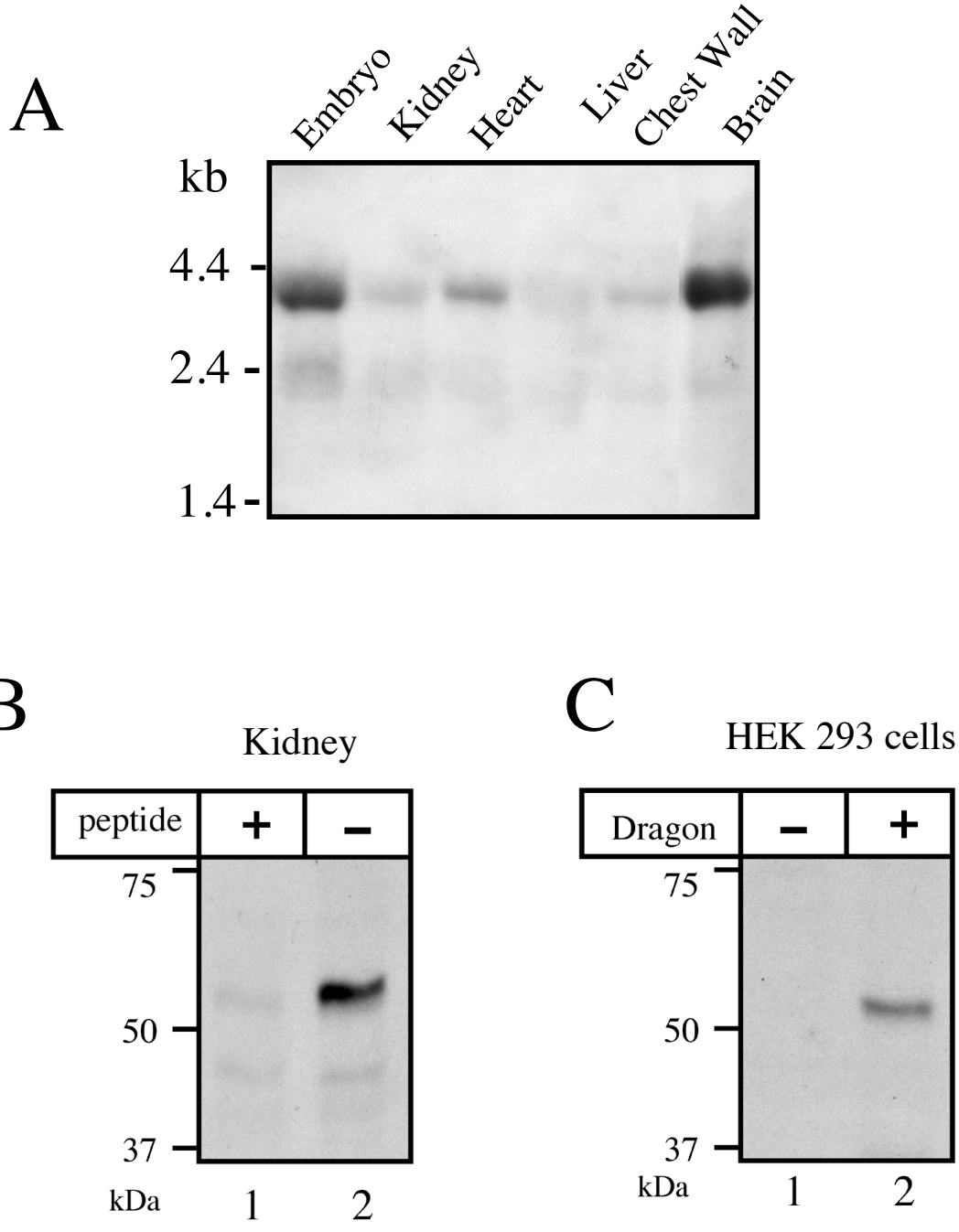
(I-K) Nuclear staining of phospho-Smad1/5/8 in distal convoluted tubules/connecting segments. Mouse kidney sections were immunostained with rabbit anti-p-Smad1/5/8 antibody (I) and mouse anti-Calbindin 28 (J). Sections were then stained with Cy3-donkey anti-rabbit (red) and FITC-donkey anti-mouse (green) secondary antibodies to visualize phospho-Smad1/5/8 and Calbindin. Merged image is shown in panel K. (L-N) Nuclear staining of phospho-Smad1/5/8 in thick ascending limbs. Mouse kidney sections were immunostained with rabbit anti-p-Smad1/5/8 antibody (L) and goat anti- anti-Tamm-Horsfall glycoprotein (α-THP, M). Sections were then stained with Cy3-donkey anti-rabbit (red) and FITC-donkey anti-goat (green) secondary antibodies to visualize phospho-Smad1/5/8 and THP. Merged image is shown in panel N.

Supplemental Figure 4. Specificity and efficacy of siRNAs targeting BMP2, BMP4, BMP6 and BMP7 expression. siRNAs (60 nM) derived from sequences of BMP2, BMP4, BMP6 and BMP7 were employed to decrease their expression in IMCD3 cells. mRNA levels were measured 46 h after cells were transfected with specific siRNA by quantitative real-time PCR, were normalized to RPL19 mRNA levels, and are expressed as a fraction of values from cells treated with negative control siRNA. The values shown are the means ± S.E. of triplicate measurements. *, $p < 0.05$, **, $p < 0.01$, and ***, $p < 0.001$ vs control siRNA.

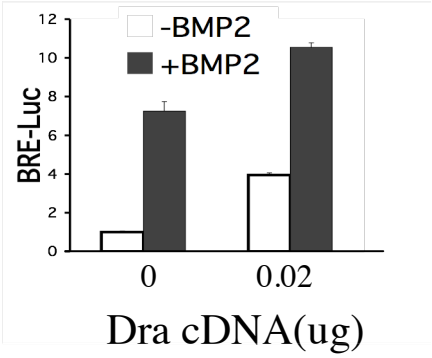
Supplemental Figure 5. Expression of BMP type II receptors in IMCD3 cells and specificity and efficacy of siRNAs targeting BMPRII, ActRIIA, and ActRIIB expression. (A) Total RNA from IMCD3 cells was extracted for RT-PCR to determine the expression of mouse BMPRII, ActRIIA, and ActRIIB. Reactions without cDNA were used as negative controls. (B) siRNA-mediated specific inhibition of BMPRII, ActRIIA, and ActRIIB expression. siRNAs (60 nM) derived from sequences of BMPRII, ActRIIA, and ActRIIB were employed to decrease their expression in IMCD3 cells. mRNA levels were measured 46 h after cells were transfected with specific siRNA by quantitative real-time PCR, were normalized to RPL19 mRNA levels, and are expressed as a fraction of values from cells treated with control siRNA. **, $p < 0.01$, and ***, $p < 0.001$ vs control siRNA.

Supplemental Figure 6. (A) IMCD3 cell numbers 3 days after initiation of BMP4 treatment. (B) Phosphorylation of SMAD1/5/8 and MAPK p38 in mIMCD3 cells detected by immunoblot after pretreatment with LDN-193189 for 30 min followed by treatment with BMP4 for 45 min. Equivalent protein loading was confirmed by detection of total SMAD1 and total MAPK p38. (C) IMCD3 cell numbers 3 days after Dragon transfection. *, $p < 0.05$, **, $p < 0.01$ vs untreated cells.

Supplemental Figure 1



Supplemental Figure 2



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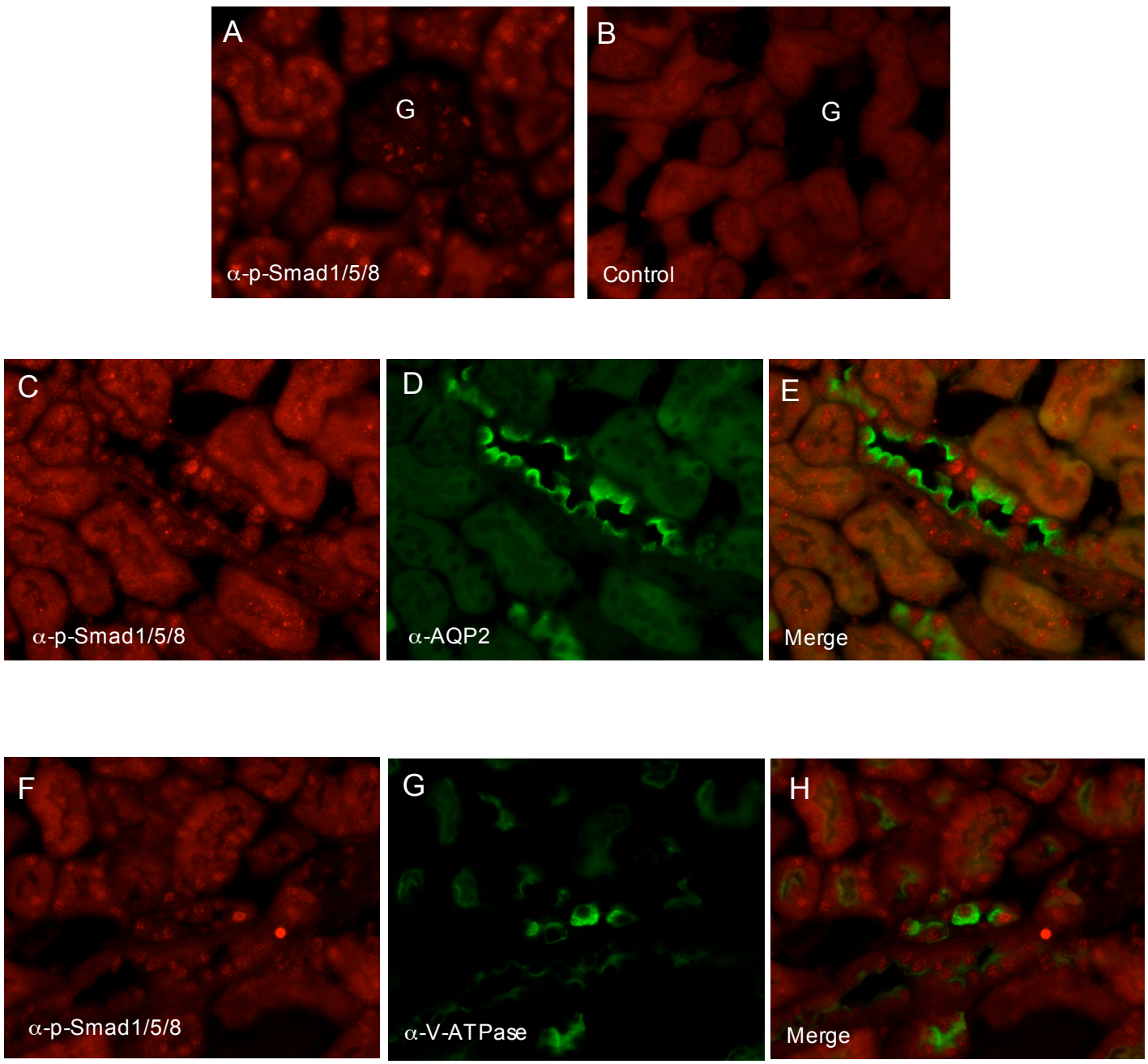

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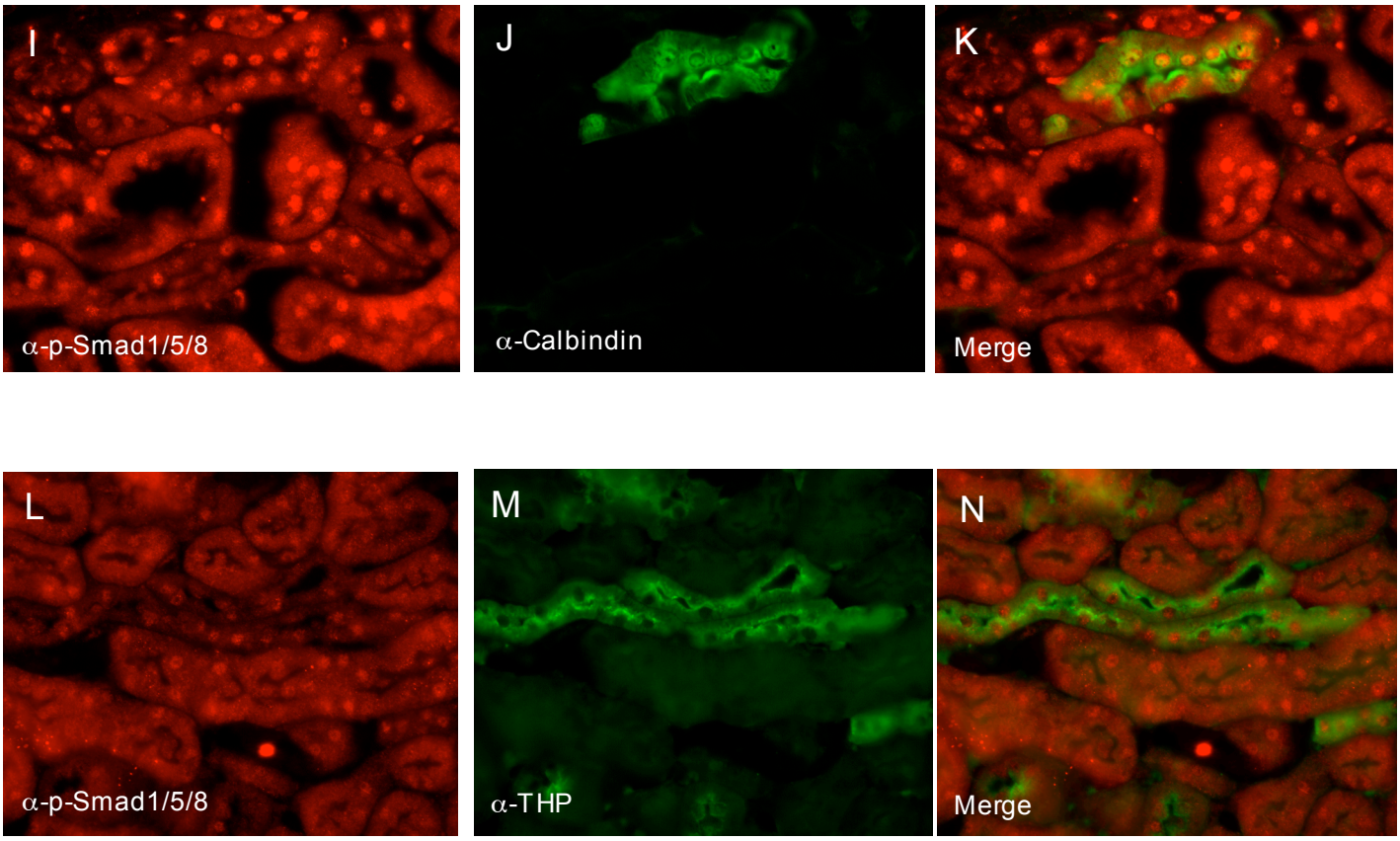

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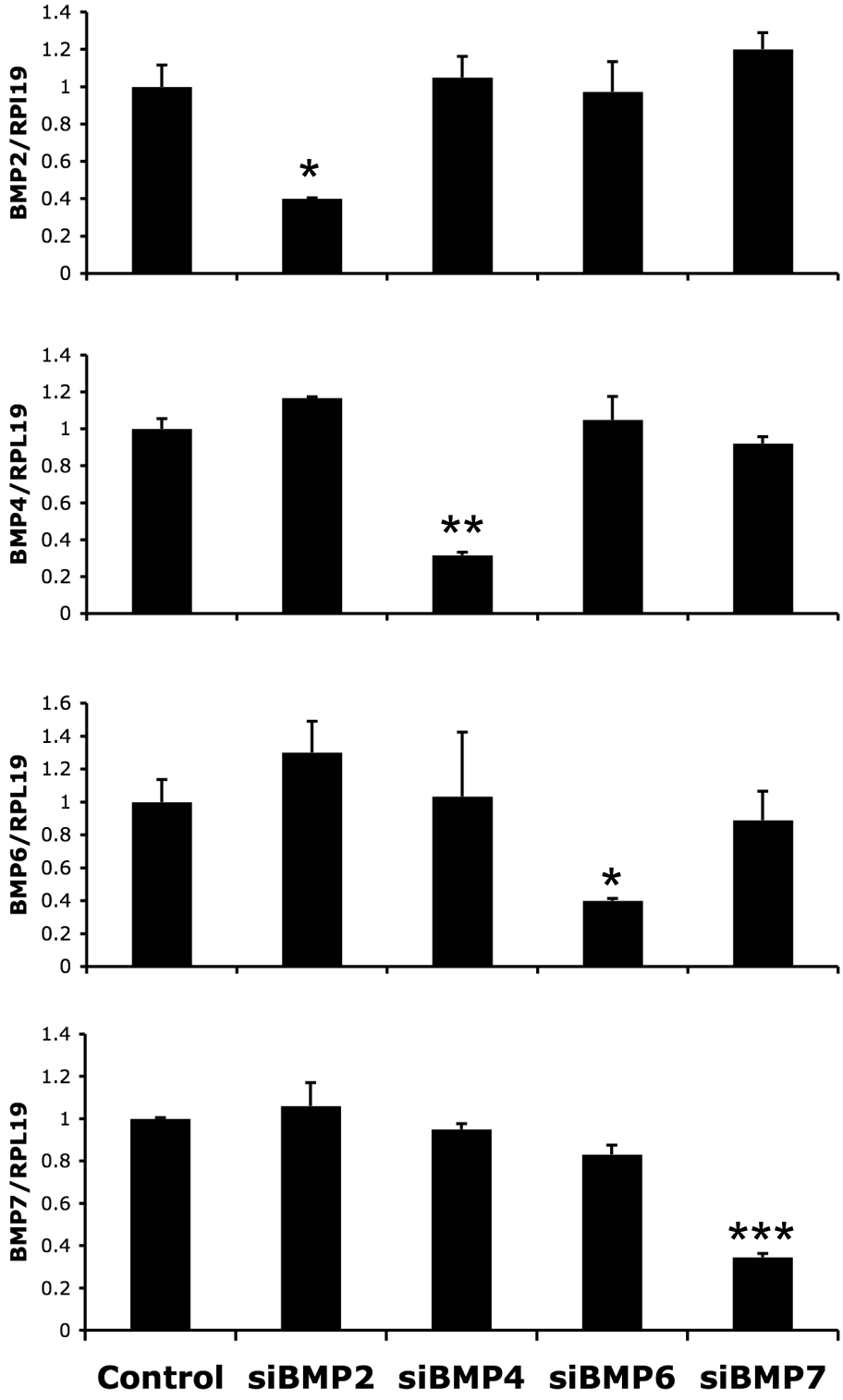

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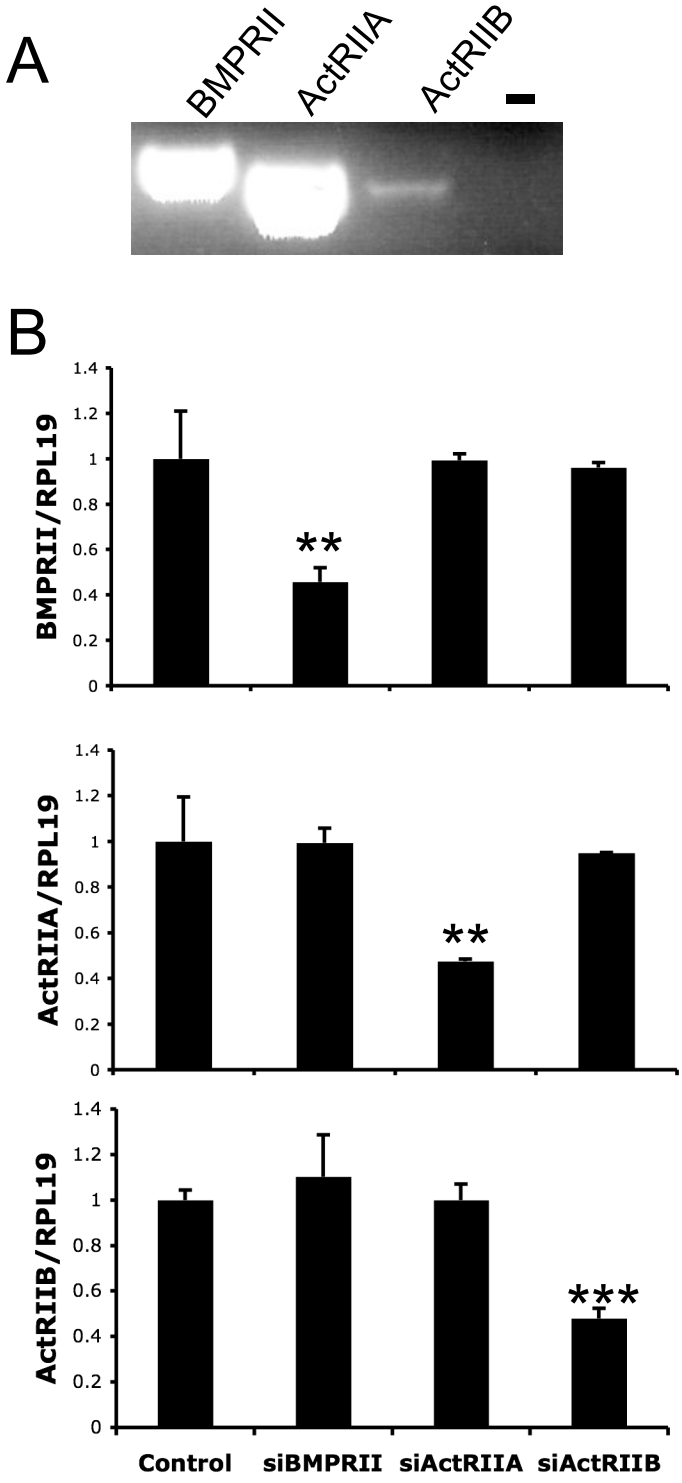
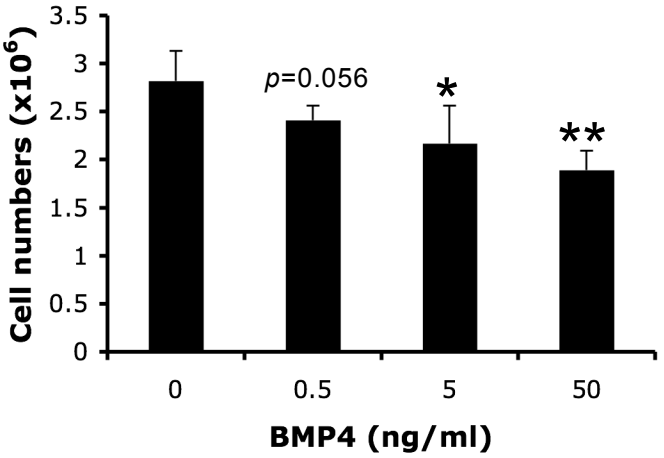


Supplemental Figure 6

A



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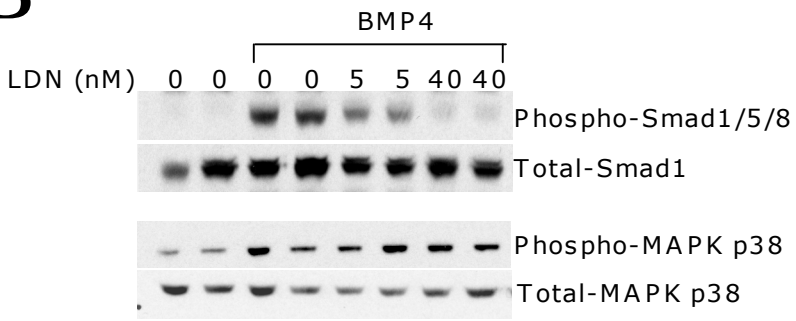

C