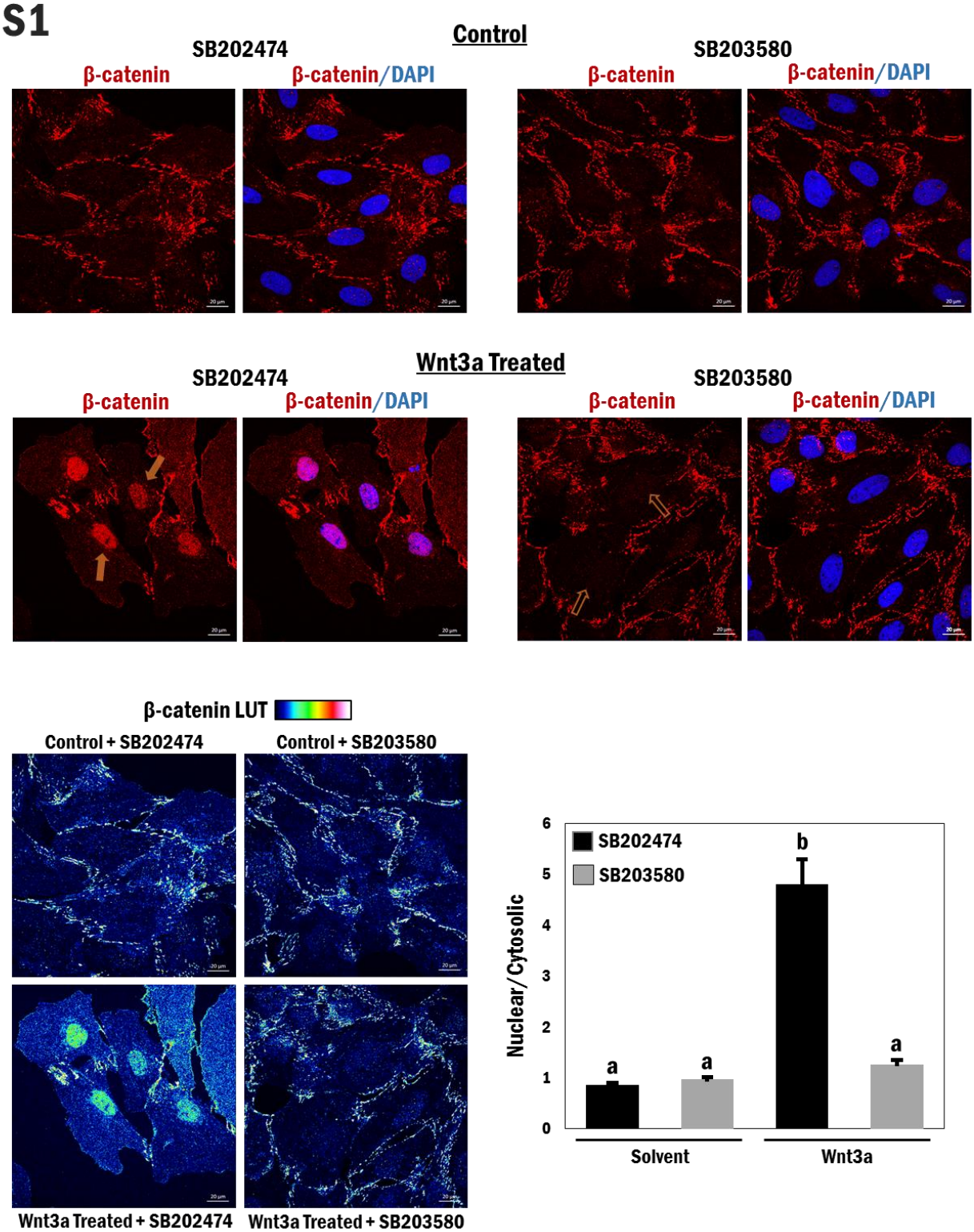
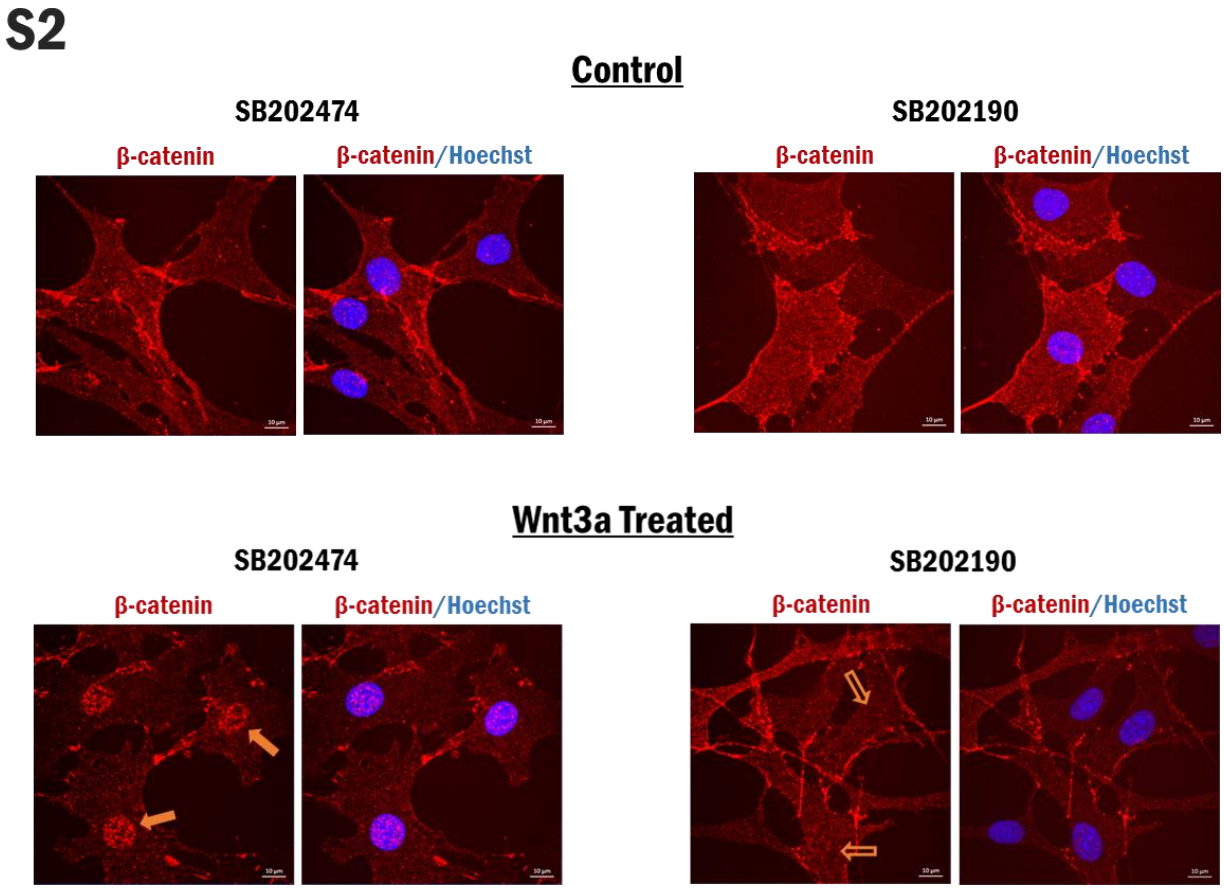
Supplementary Figures

S1

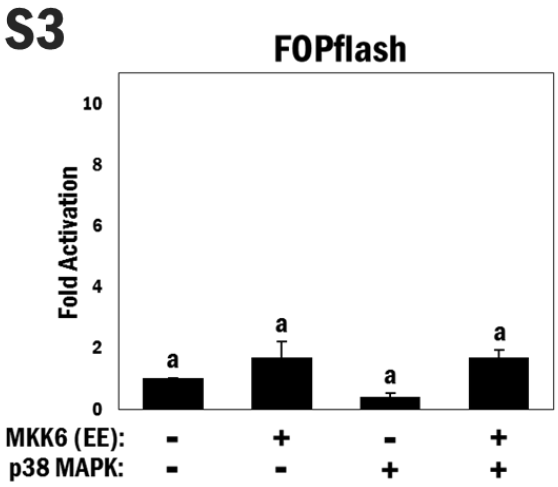


S1. Modulation of β-catenin localization with Wnt3a stimulation and p38 MAPK inhibition. A10 cells were serum starved for 12 hr and pre-treated with SB203580 or SB202474 (10 μM, 45 min) then stimulated with Wnt3a (200 ng/mL) or solvent (PBS, control) for 4 hr. Cells were fixed and stained with DAPI (blue, nucleus) and TRITC (red, β-catenin). Filled arrows indicate presence of nuclear β-catenin, empty arrows indicate lack thereof. (Bottom left panel) A rainbow (blue to red) lookup table (LUT) was used to indicate relative β-catenin intensity. (Bottom right panel) Relative nuclear to cytosolic fluorescence levels were measured and quantified (n=28, 25, 21, 27 from left to right); b=p≤0.0001.

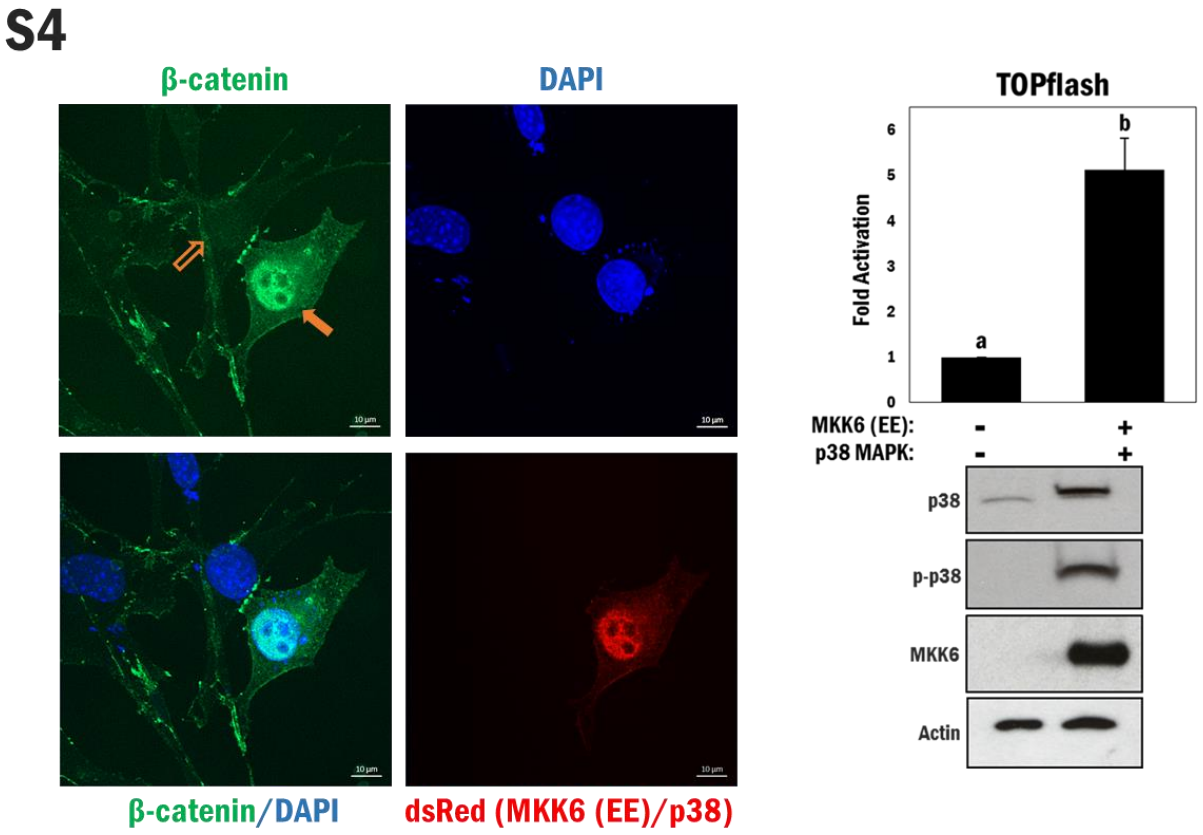


S2. Modulation of β-catenin localization with Wnt3a stimulation and p38 MAPK inhibition. Primary VSMCs were serum starved for 12 hr and pre-treated with SB202190 or SB202474 (10

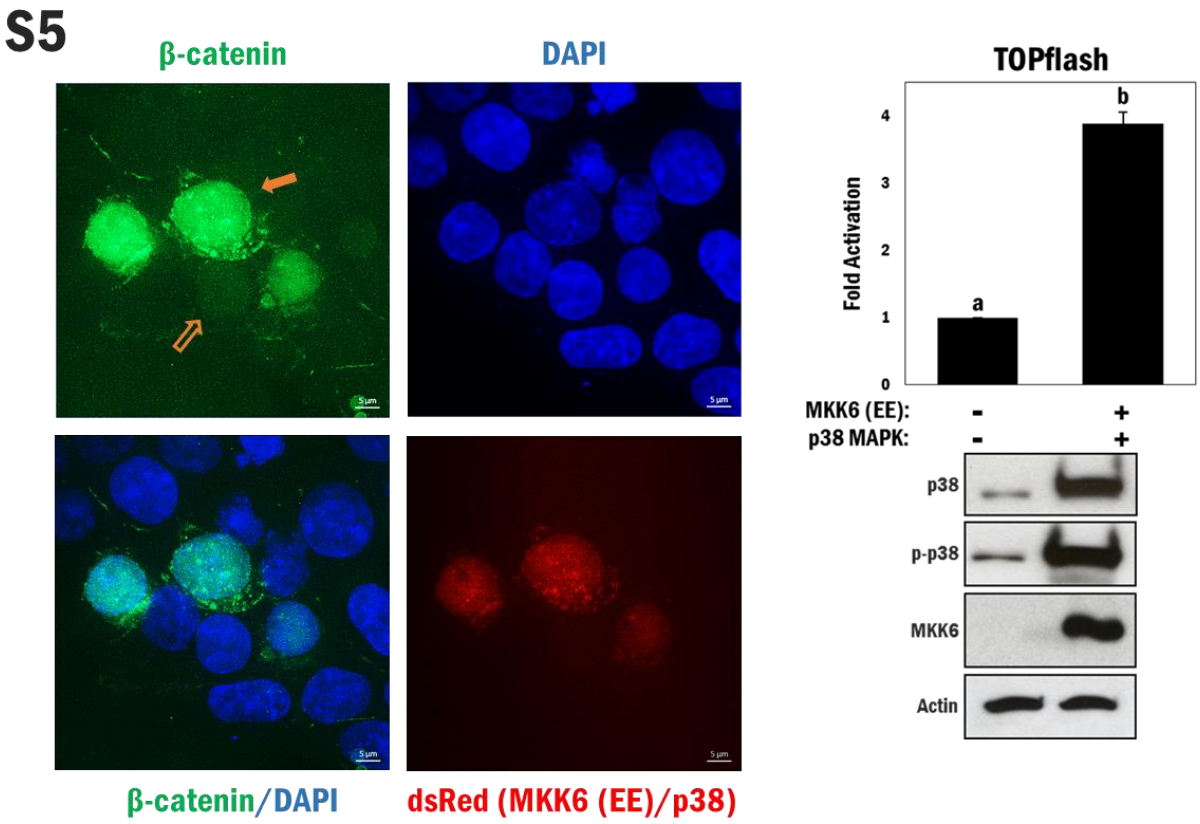
μM, 45 min) then stimulated with Wnt3a (200 ng/mL) or solvent (PBS, control) for 4 hr. Cells were fixed and stained with DAPI (blue, nucleus) and TRITC (red, β-catenin). Filled arrows indicate presence of nuclear β-catenin, empty arrows indicate lack thereof.



S3. FOPflash reporter gene activity in response to active p38 MAPK signalling. FOPflash reporter gene activity was measured in A10 cells that were transfected with different combinations of p38 MAPK and active MKK6 (EE) as indicated.

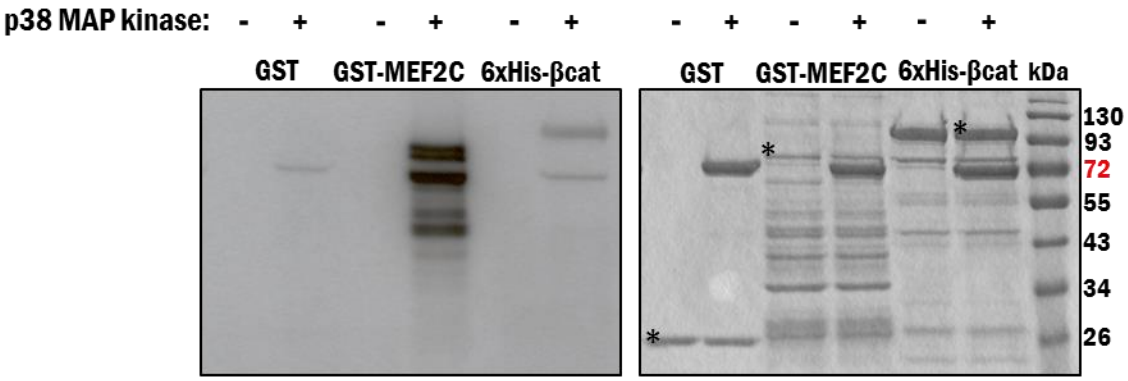


S4. β-catenin localization and *trans*activation in response to active p38 MAPK. (Left panel) 10T1/2 cells were co-transfected with MKK6 (EE) and p38 MAPK and marked for ectopic expression (dsRed, red), DAPI was used for nuclear staining and FITC was used for β-catenin staining (green); (Top right panel) TOPflash reporter gene activity was measured in 10T1/2 cells that were transfected with different combinations of p38 MAPK and active MKK6 (EE) as indicated; b=p≤0.05. (Bottom right panel) Western blot analysis to show activation of p38 MAPK.

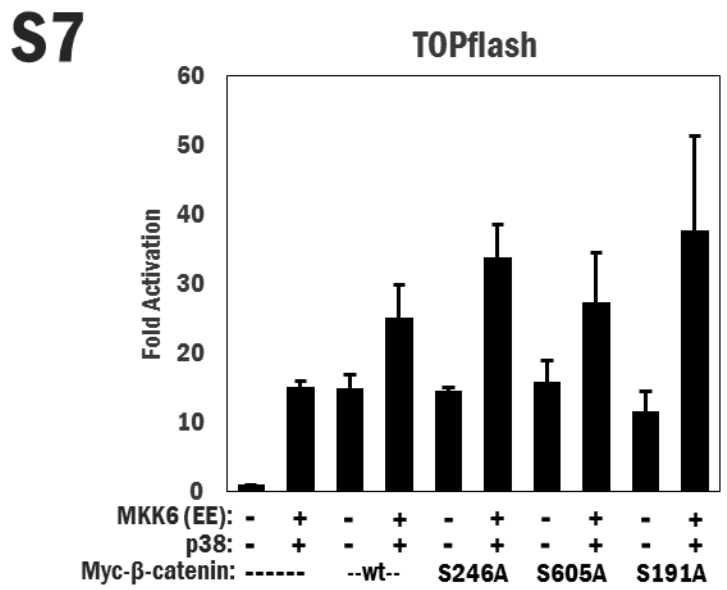


S5. β-catenin localization and *trans*activation in response to active p38 MAPK. (Left panel) HEK 293T cells were co-transfected with MKK6 (EE) and p38 MAPK and marked for ectopic expression (dsRed, red), DAPI was used for nuclear staining and FITC was used for β-catenin staining (green); (Top right panel) TOPflash reporter gene activity was measured in HEK 293T cells that were transfected with different combinations of p38 MAPK and active MKK6 (EE) as indicated; b=p≤0.01. (Bottom right panel) Western blot analysis to show activation of p38 MAPK.

S6

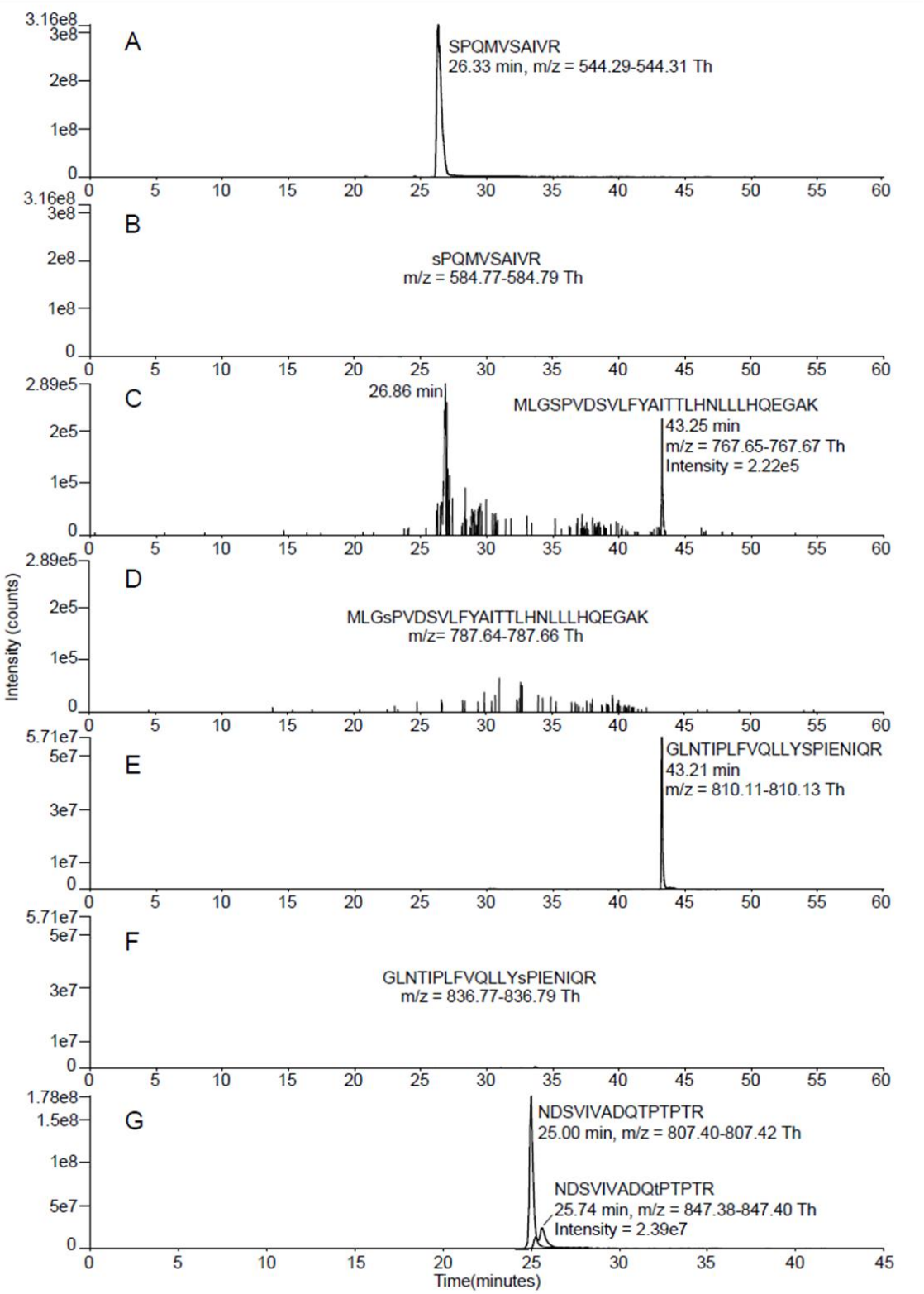


S6. Determination of β-catenin as a potential substrate of p38 MAPK by *in vitro* kinase assay. Purified p38 MAPK was incubated with [$^{\gamma\text{-}32}$P]ATP, and either GST (negative control), GST-MEF2C (positive control), or 6xHis-β-catenin, as indicated, for 30 min at 30°C. The subsequent SDS-PAGE was exposed to X-ray film to determine ^{32}P incorporation. Stars indicate location of substrate proteins.

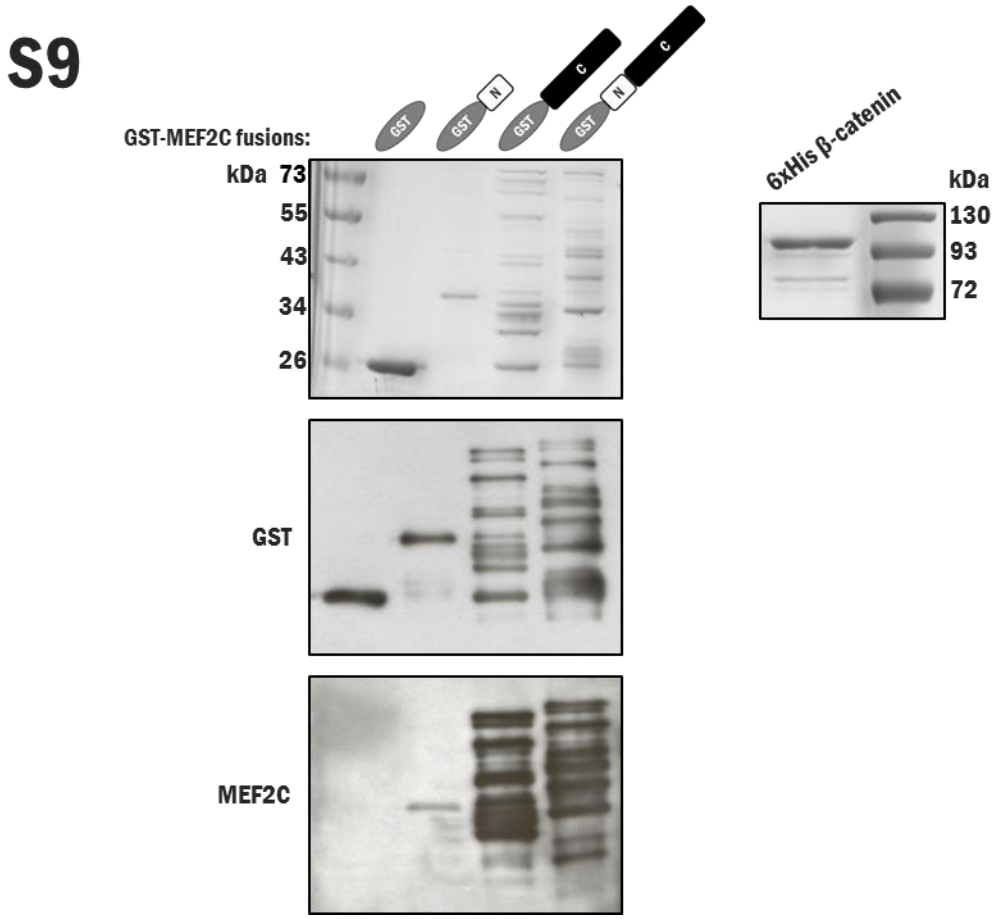


S7. Mutational analysis of β-catenin transactivation properties following Ser to Ala mutations of putative MAPK phospho-sites in response to p38 MAPK signalling. Wild-type or mutated Myc-tagged β-catenin was over-expressed with MKK6 (EE) and p38 MAPK in a TOPflash reporter assay, as indicated.

S8

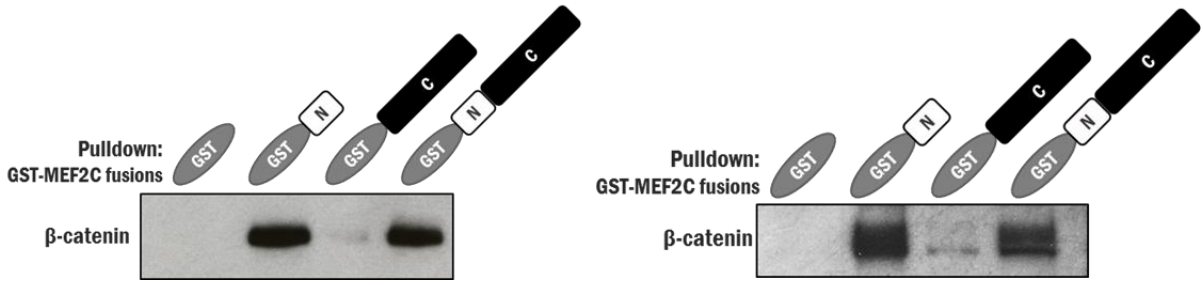

A
SPQMVSAIVR
26.33 min, m/z = 544.29-544.31 Th
B
sPQMVSAIVR
m/z = 584.77-584.79 Th
C
26.86 min
MLGSPVDSVLFYAITTLHNLLLHQEGAK
43.25 min
m/z = 767.65-767.67 Th
Intensity = 2.22e5
D
MLGsPVDSVLFYAITTLHNLLLHQEGAK
m/z= 787.64-787.66 Th
E
GLNTIPLFVQLLYSPIENIQR
43.21 min
m/z = 810.11-810.13 Th
F
GLNTIPLFVQLLYsPIENIQR
m/z = 836.77-836.79 Th
G
NDSVIVADQTPTPTR
25.00 min, m/z = 807.40-807.42 Th
NDSVIVADQtPTPTR
25.74 min, m/z = 847.38-847.40 Th
Intensity = 2.39e7
Intensity (counts)
Time(minutes)

S8. Ion chromatograms of tryptic β-catenin peptides containing candidate p38 MAPK phosphosites and a tryptic ATF2 peptide and phosphopeptide containing known p38 MAPK phosphosites. Phosphorylated residues are shown as lower case letters. Doubly protonated SPQMVSAIVR (A) from β-catenin gave a prominent chromatographic peak while the corresponding phosphopeptide (B) did not. The quadruply protonated peptide MLGSPVDSVLFYAITTLHNLLLHQEGAK (C) was confirmed by MS/MS at 43.25 minutes while the ion at 26.86 minutes was an isobaric species. No chromatographic peak from phosphorylated MLGSPVDSVLFYAITTLHNLLLHQEGAK was present (D). Doubly protonated GLNTIPLFVQLLYSPIENIQR (E) was observed at 43.21 minutes while the extracted ion chromatogram of the phosphorylated counterpart (F) had no signal. The ATF2 peptide NDSVIVADQTPTPTR is shown in both its unmodified and phosphorylated state in panel G.



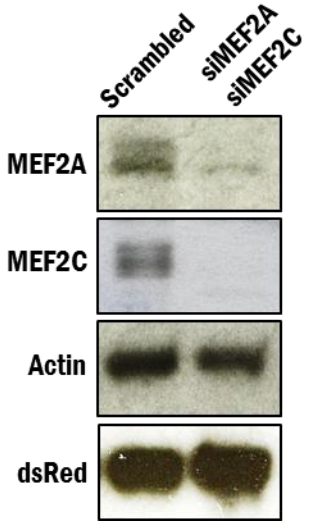
S9. Purification of GST-MEF2C and 6xHis-β-catenin. GST-MEF2C or 6xHis-β-catenin fusion proteins were purified from *E. coli* using GST-agarose or Nickel-agarose columns, respectively. Representative Coomassie blue SDS-PAGE and Western blots are shown to confirm purification.

S10



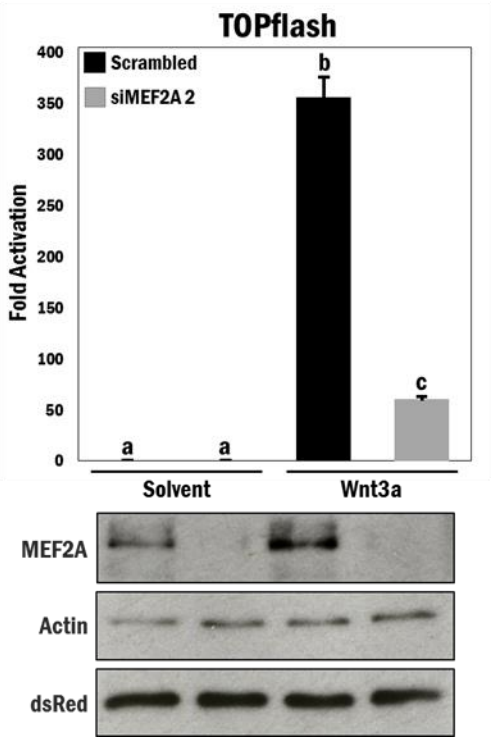
S10. *In vitro* binding assay. An *in vitro* GST pull down assay was performed using purified 6x-His-β-catenin and either GST (control), the N-terminal (aa 1-86), C-terminal (aa 87-465), or full length (aa 1-465) GST-MEF2C fusion proteins.

S11



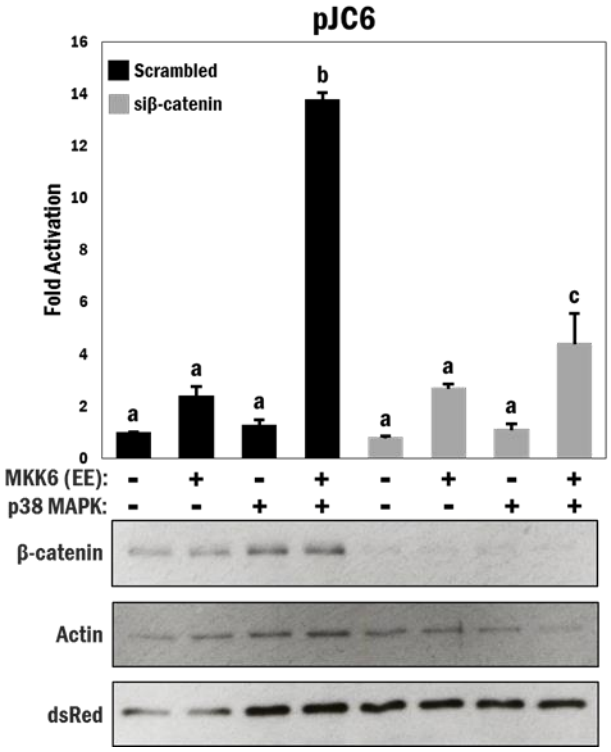
S11. Confirmation of siRNA mediated silencing of MEF2A/C. A10 cells were transfected with either scrambled RNA control or siRNA for MEF2A and MEF2C, and confirmed by Western analysis.

S12

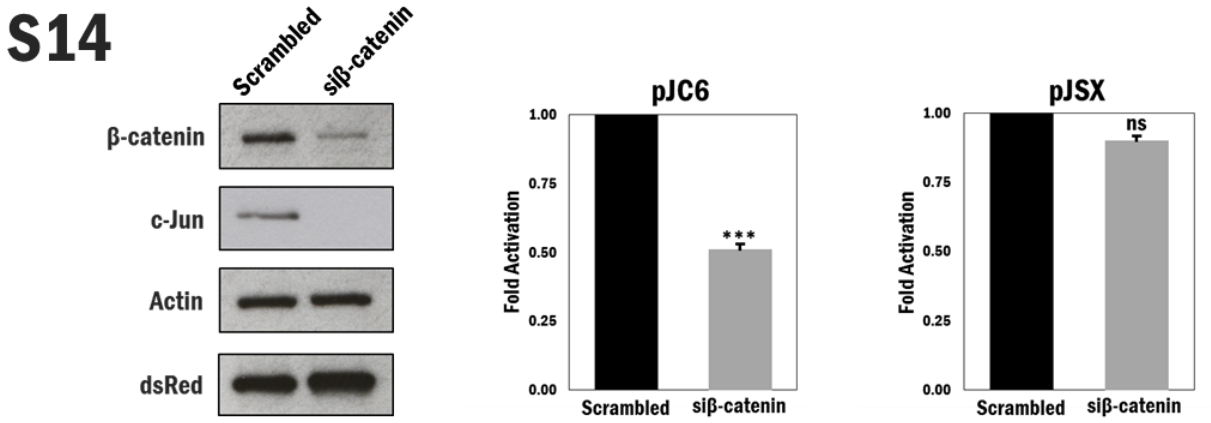


S12. TOPflash assay during Wnt stimulation using siRNA to MEF2A. (Top panel) A10 cells were transfected with TOPflash reporter, scrambled or siMEF2A RNA for 3 hr, cultured in serum conditions for 24 hr followed by treatment with either Wnt3a (200 ng/mL) or solvent (PBS) for 16 hr in serum free media; b,c=$p \leq 0.0001$. (Bottom panel) Corresponding Western blot analysis of cell lysates to confirm siRNA-mediated MEF2A depletion.

S13



S13. c-Jun reporter assay with active p38 MAPK and β-catenin gene silencing. c-Jun reporter gene (pJC6) activity was measured in A10 cells under various combinations of ectopically expressed MKK6 (EE) and p38 MAPK, and either scrambled RNA (control) or siRNA β-catenin, as indicated in the figure; b,c=$p \leq 0.0001$.



69 **S14.** Western blot analysis of A10 cells that have been transfected with scrambled shRNA

70 (control) or shRNA β-catenin with comparative pJC6 and pJSX reporter activities; ***=p≤0.001.