Supplementary Figures

Supplementary Figure 1. NIPA1 co-localises with endosomal markers in HeLa and NSC34 cells. Stably expressed NIPA1-GFP co-localises in HeLa cells with endogenous markers of recycling endosomes (transferrin receptor; TFNR; A) and retromer-positive endosomes (VPS26; B). Stably expressed NIPA1-GFP co-localises in NSC34 cells with endogenous markers of early endosomes (EEA1; C, arrows indicate co-localised puncta) and lysosomes (Lamp1; D). In these and subsequent supplementary micrographs the right hand panels show the merged images; the colour of each marker in the merged image is shown by the colour of its lettering in the non-merged panels. Scale bars=10 μm in these and subsequent micrographs.

Supplementary Figure 2. BMP signaling assays in control cells and using individual NIPA1 siRNA oligonucleotides. A) A representative immunoblot showing the pSmad1/5 response to BMP stimulation in mock-transfected cells or cells depleted of BMPRII by siRNA. The corresponding histogram shows quantitation of the pSmad1/5 immunoblot density in 3 such experiments. B) A representative immunoblot showing pSmad1/5 levels in mock-transfected HeLa cells, or in cells depleted of NIPA1 by siRNA knock-down (KD) using a pool of 4 siRNA oligonucleotides, or siRNA1 or siRNA2 oligonucleotides individually. Cells were not stimulated with BMP4. The results of 4 experiments using the individual oligonucleotides are quantified in the histogram, normalized against the mock-transfected value. C) The effect of NIPA1 depletion by siRNA KD using individual oligonucleotides siRNA1 or siRNA2 on *Id* luciferase reporter gene activation, normalized to *Renilla* luciferase activity and protein mass, on unstimulated cells and cells stimulated with the indicated dose of BMP4 (n= 6). D) A histogram of *Id* luciferase reporter gene activation, normalized as in C), in mock-transfected HeLa cells and HeLa cells depleted of BMPRII by siRNA (n=4). E) A representative immunoblot showing pSmad1/5 levels in wild-type (WT) NSC34 cells, or in NSC34 cells stably expressing GFP-Rab5. The corresponding histogram shows quantitation of the pSmad1/5 immunoblot density in 3 such experiments. F) A histogram of *Id* luciferase reporter gene activation, normalized as in C), in wild-type NSC34 cells versus NSC34 cells stably expressing GFP-Rab5, n=3. In A, C, D, E and F), graphed values were normalized by dividing by the value for stimulated WT or depletion-control value. In A), B) and E) actin immunoblotting is shown to verify equal loading. Error bars=s.e.m., p-values were calculated using two-tailed paired t-tests.

Supplementary Figure 3. NIPA1-GFP promotes internalization of BMPRII to endosomes. Wild type HeLa cells (A, C) or HeLa cells stably transfected with NIPA1-GFP (B, D) were transiently transfected with myc-BMPRII and labeled with the late endosomal marker M6PR (A, B) or the recycling endosomal marker TFNR (C, D). In the NIPA1-GFP expressing cells, myc-BMPRII is substantially redistributed from the plasma membrane to internal vesicles that co-label with the endosomal markers. Arrows point to individual vesicles that show co-localisation.

Supplementary Figure 4. NIPA1-GFP does not alter the distribution or concentration of EGFR. A) In wild-type HeLa cells, endogenous EGFR is predominantly present on the plasma membrane. B) The distribution of EGFR does not alter in HeLa cells stably transfected with NIPA1-GFP. C) A representative immunoblot in which lysates from wild-type HeLa cells or HeLa cells stably expressing NIPA1-GFP have been immunoblotted for EGFR. Actin immunoblotting is shown to verify equal loading. The histogram shows quantitation of the EGFR immunoblots for 3 such experiments, normalized against wild-type levels. Error bars=s.e.m.

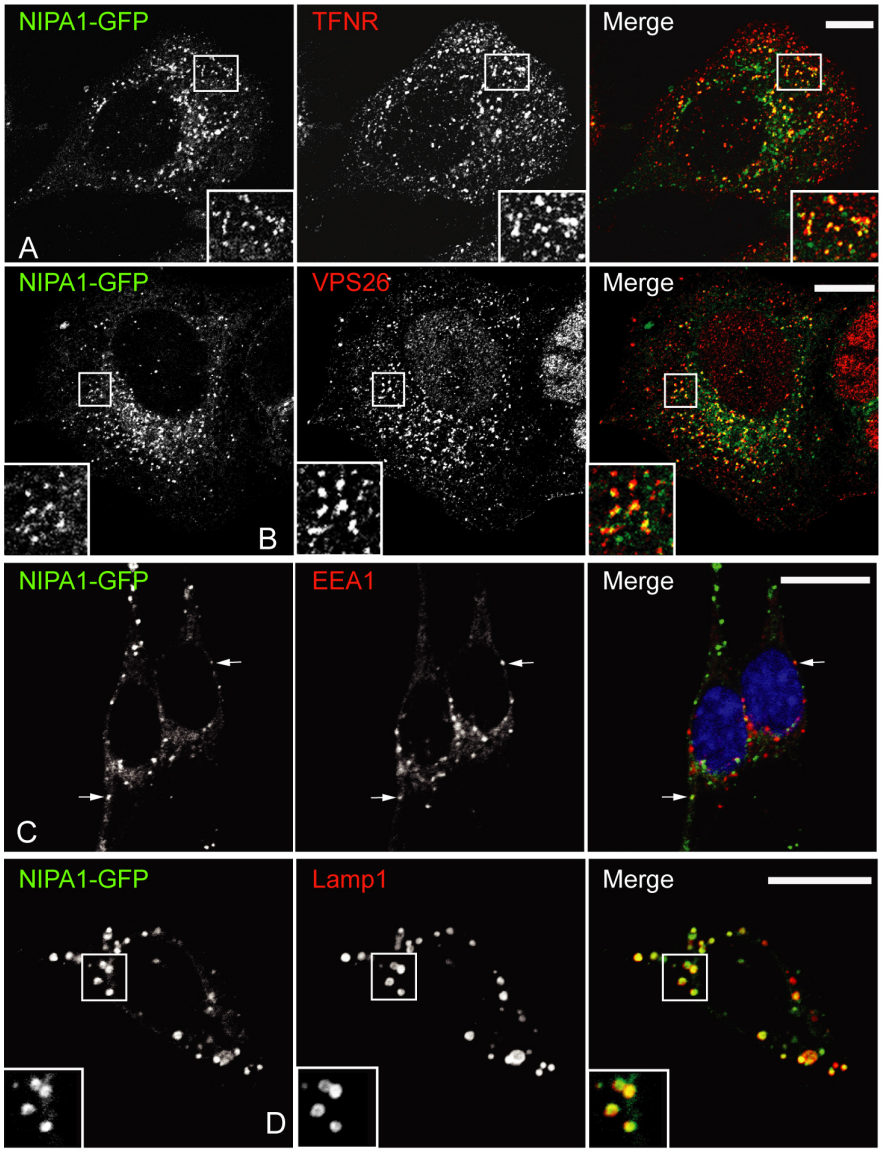
Supplementary Figure 5. Effects of depletion of NIPA1 on BMPRII levels, using individual siRNA oligonucleotides. A) A representative immunoblot showing endogenous BMPRII levels in mock-transfected HeLa cells, or in cells depleted of NIPA1 by siRNA KD using a pool of 4 siRNA oligonucleotides, or siRNA1 or siRNA2 individually. The corresponding histogram shows the results of 5 experiments using the individual oligonucleotides, normalized against the mock-transfected value. Error bars=s.e.m., p-values were calculated using two-tailed paired t-tests.

Supplementary Figure 6. Subcellular distribution of mutant NIPA1. In HeLa cells stably transfected with G106R NIPA1-GFP, NIPA1 could be distributed in a reticular pattern, when it showed co-localisation with the ER marker calreticulin (A). In other cells, the mutant NIPA1 was punctate, and co-localised with endosomal markers, including Lamp1 (B and not shown). In B), arrows indicate co-localised puncta. Similar results were obtained with the T45R mutant (not shown).

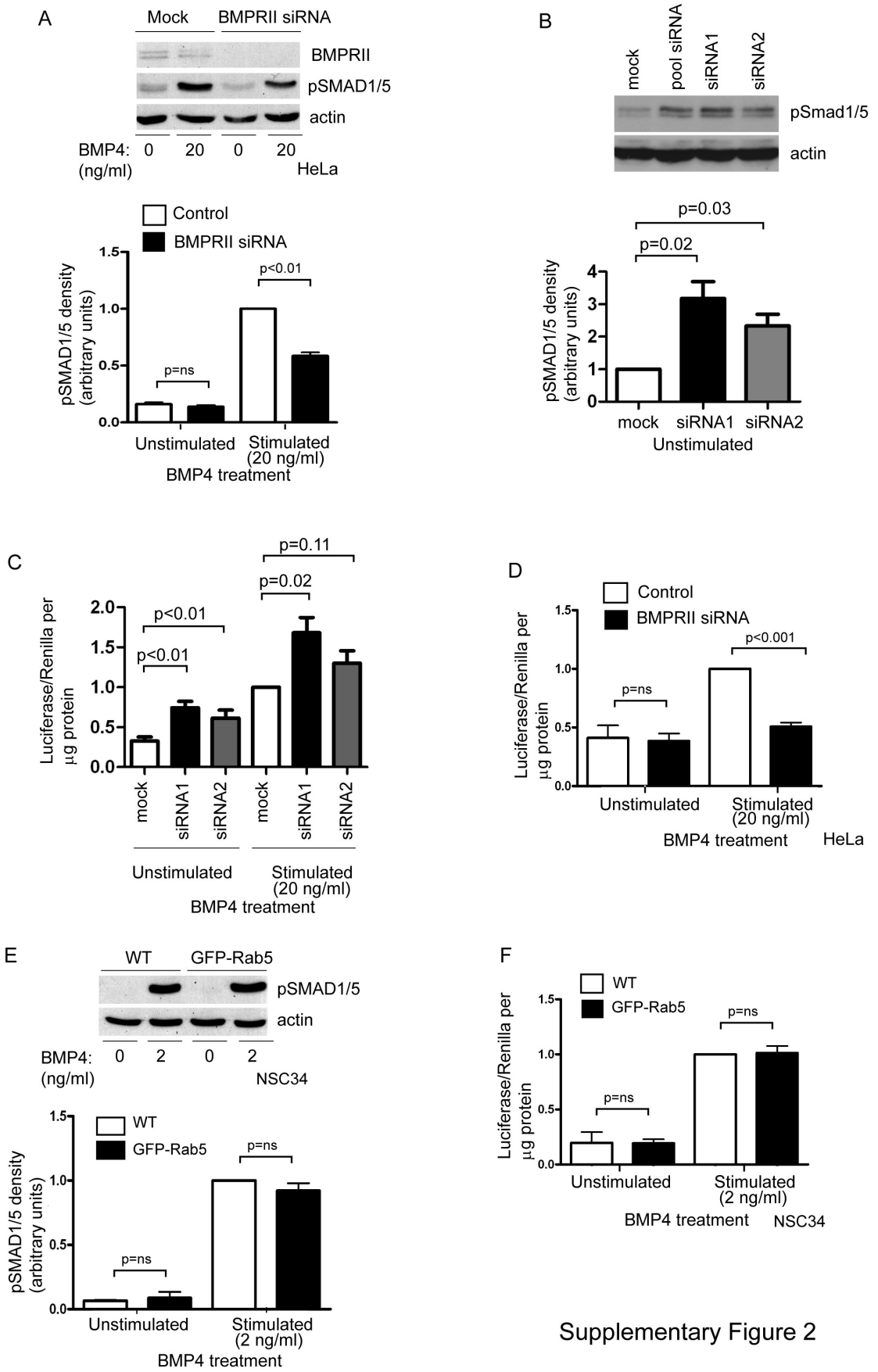
Supplementary Figure 7. Effect of mutant NIPA1 on BMP signaling. (A) A representative experiment examining the effect of BMP4 stimulation on the amount of pSmad1/5, in untransfected NSC34 cells or in NSC34 cells stably expressing wild-type (WT) or mutant forms of NIPA1-GFP. Actin immunoblotting is shown to verify equal loading; GFP immunoblotting shows equal expression levels of WT and mutant NIPA1-GFP in the cell lines. The histogram shows quantitation of pSmad1/5 immunoblot band density in 3 such experiments. (B) *Id* luciferase reporter gene activity, normalized to *Renilla* luciferase activity and protein mass, in untransfected NSC34 cells or in NSC34 cells stably expressing WT or mutant forms of NIPA1-GFP, either unstimulated or stimulated with 2ng/ml BMP4 (n= 4). Graphed values were normalized by dividing by the value for stimulated untransfected sample; error bars=s.e.m. and p-values were calculated using two-tailed paired t-tests.

Supplementary Figure 8. BMP signaling assays using individual spartin and spastin oligonucleotides. A) Representative immunoblot showing the pSmad1/5 response to BMP stimulation in control-transfected cells or cells depleted of spartin by individual siRNA2. The corresponding histogram shows quantitation of the pSmad1/5 immunoblot density in 4 such experiments, normalized against the knock-down values. B) Representative immunoblot showing the pSmad1/5 response to BMP stimulation in control-transfected cells or cells depleted of spastin by individual siRNAs 1 and 3. The corresponding histogram shows quantitation of the pSmad1/5 immunoblot density in 4 such experiments, normalized against the control values. C) The effect of spartin depletion by siRNA KD using individual oligonucleotide siRNA2 on *Id* luciferase reporter gene activation, normalized to *Renilla* luciferase activity and protein mass, on unstimulated cells and cells stimulated with the indicated dose of BMP4 (n=8 for unstimulated, n=6 for stimulated cells). Charted values have been normalized against the control unstimulated values. D) The effect of spastin depletion by siRNA KD using individual oligonucleotides siRNA1 or siRNA3 on *Id* luciferase reporter gene activation, normalized to *Renilla* luciferase activity and protein mass, on unstimulated cells and cells stimulated with the indicated dose of BMP4 (n=6). Charted values have been normalized against stimulated control values. In A and B, images corresponding to each antibody in individual experiments were obtained from the same blot, intervening spurious lanes have been removed. Actin immunoblotting is shown to verify equal loading. Error bars=s.e.m., p-values were calculated using two-tailed paired t-tests. Control cells were either mock-transfected or transfected with a non-targeting siRNA.

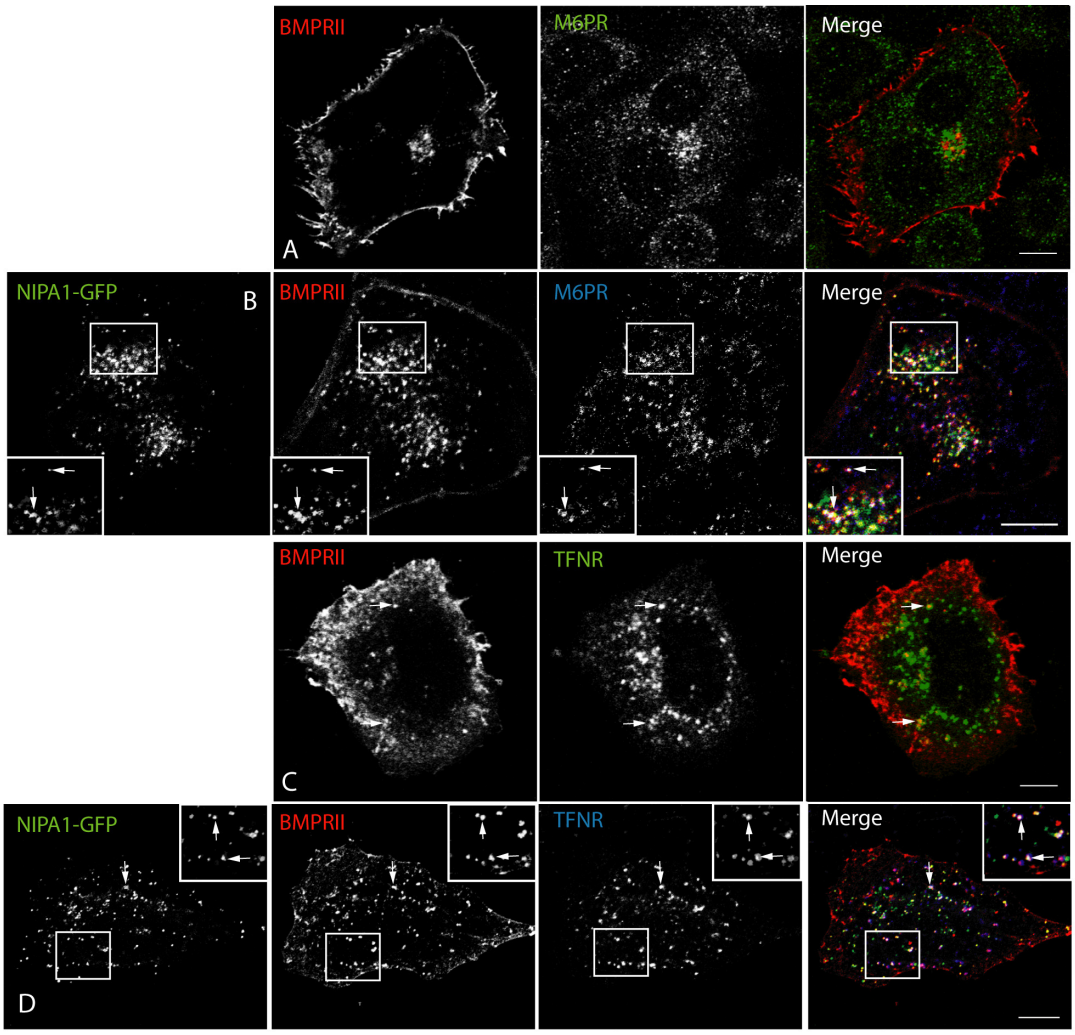
Supplementary Figure 9. Representative experiments showing methods used to verify NIPA1 depletion by siRNA. NIPA1 siRNA transfections on HeLa cells stably expressing NIPA1-GFP were carried out in parallel with, and using the same reagents as, experiments on wild-type HeLa cells. Loss of NIPA1-GFP was subsequently confirmed by immunofluorescence (A) or immunoblotting (see Fig. 1A) with anti-GFP antibody in the NIPA1-GFP cell line, and used as a reporter for NIPA1 depletion in the wild-type cells transfected with the same reagents. In A), DAPI staining is shown in blue, NIPA1-GFP in green. Alternatively or in addition, NIPA1 depletion was verified by quantitative RT-PCR in wild-type HeLa cells transfected with NIPA1 siRNA. A representative experiment is shown in B). The histogram shows the relative amount of NIPA1 mRNA in mock transfected wild-type HeLa cells, cells transfected with a pool of NIPA1 siRNA oligonucleotides 1-4, or with siRNA 1 or 2 individually. RT-PCR assays were carried out in triplicate. Error bars= 95% confidence intervals.



Supplementary Figure 1



Supplementary Figure 2



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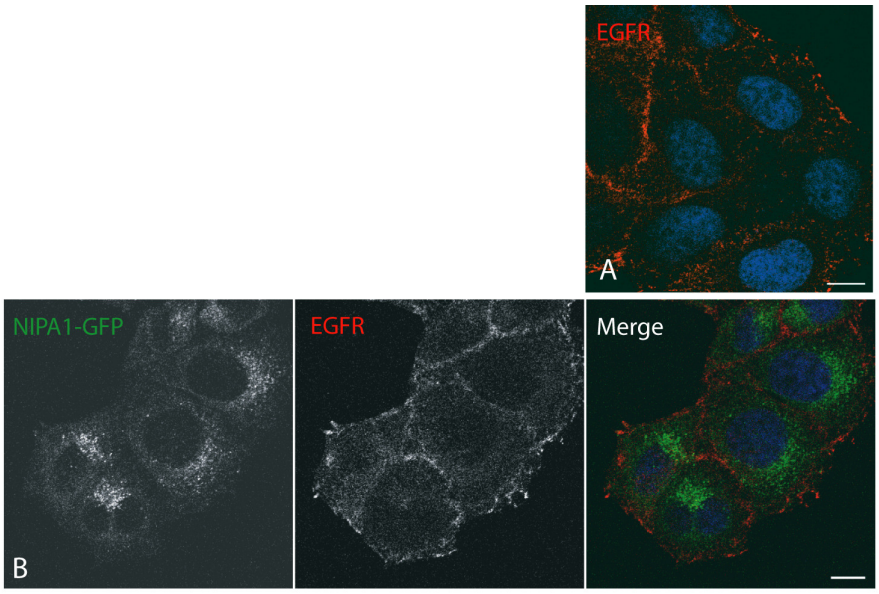
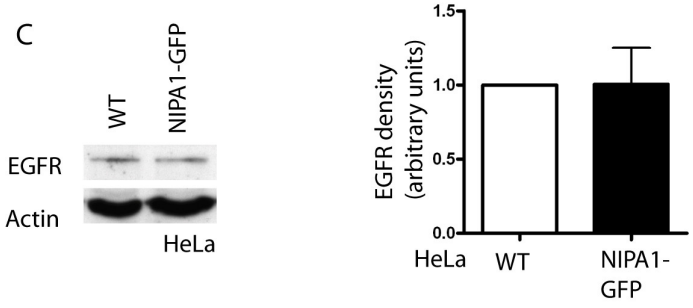
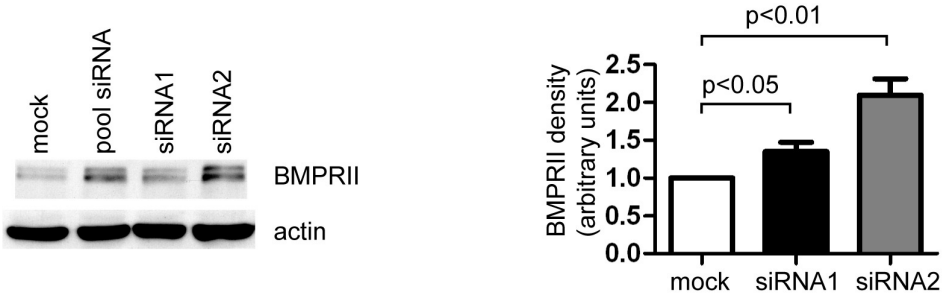


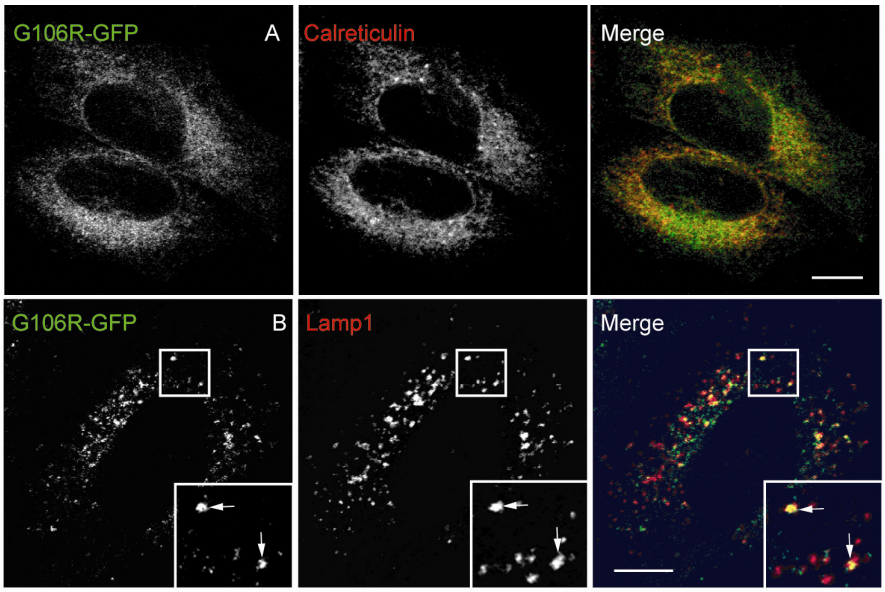


Supplementary Figure 4

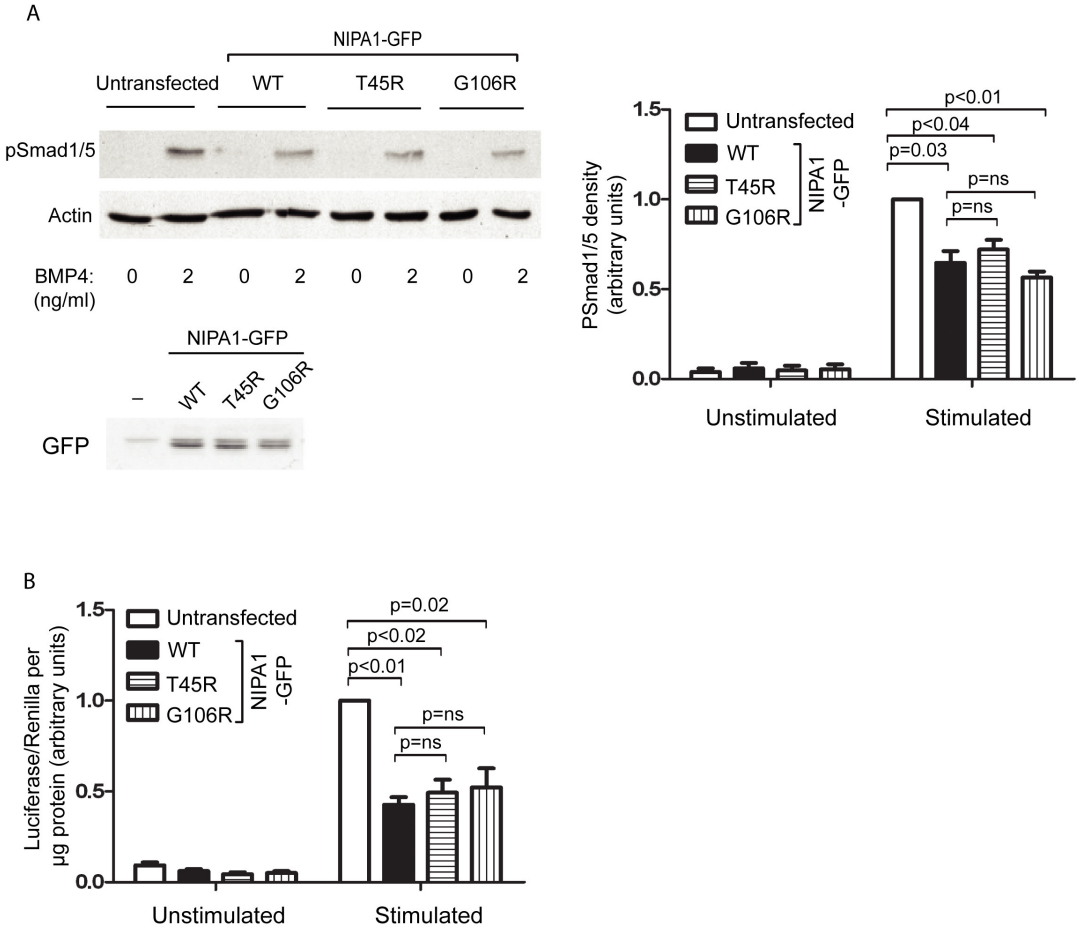
A



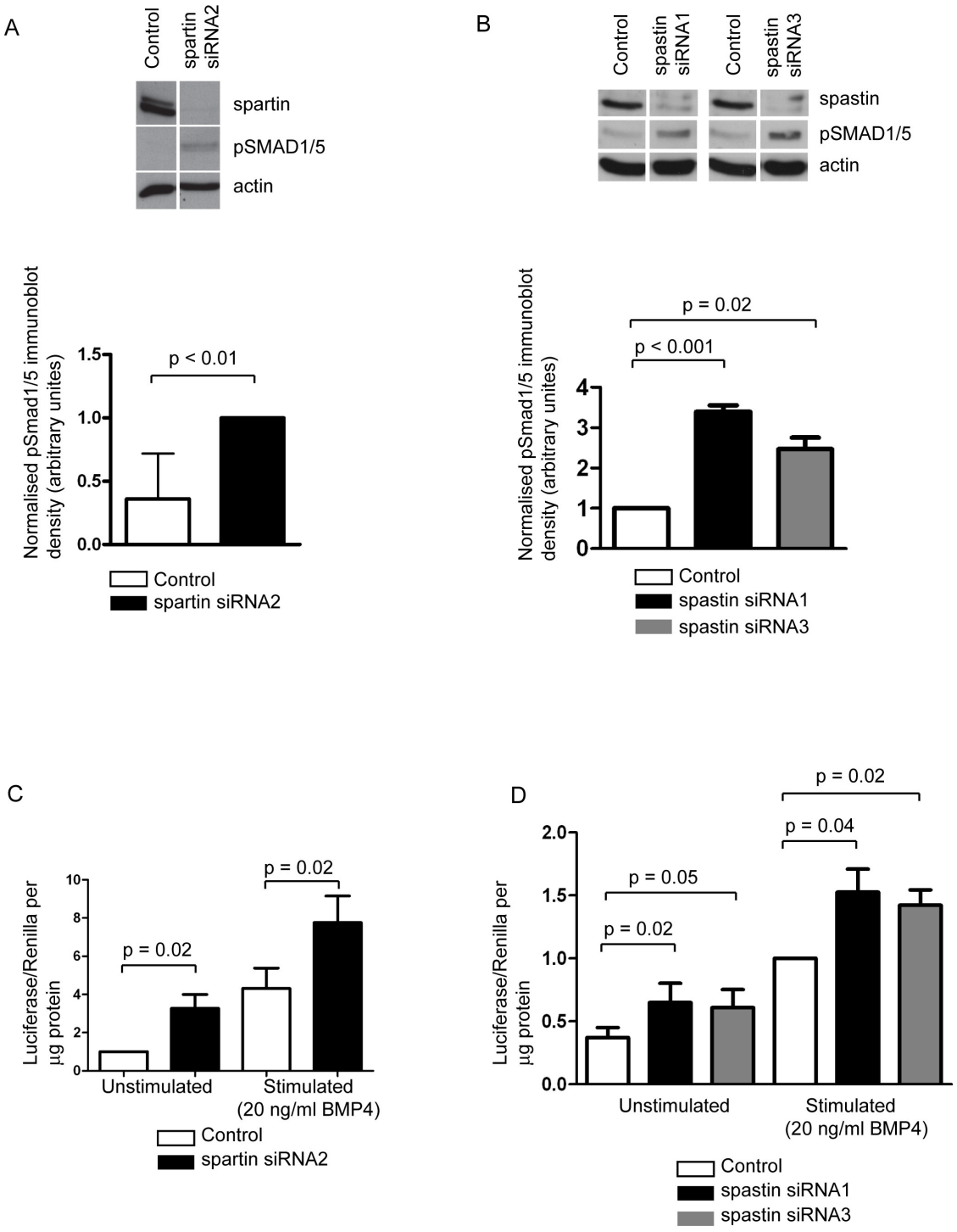
Supplementary Figure 5



Supplementary Figure 6

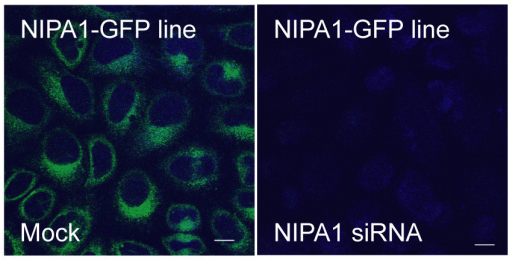


Supplementary Figure 7



Supplementary Figure 8

A



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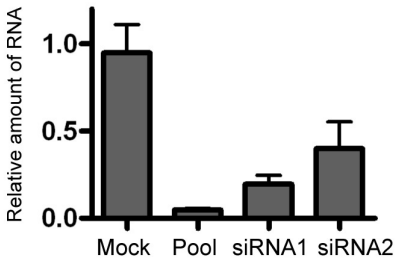

Supplementary Figure 9