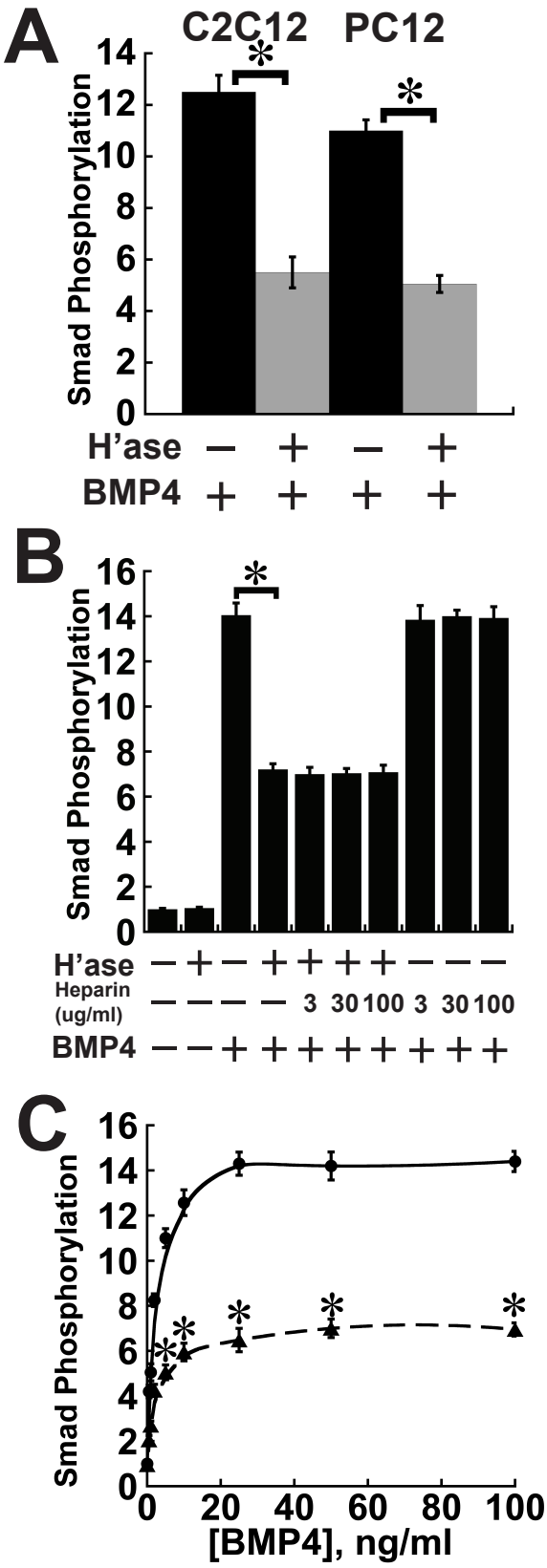
Supplemental Figure 1. Heparitinase treatment diminishes responses of cultured cells to BMP4. (**A**) Smad phosphorylation in mouse C2C12 and rat PC12 cells. Cells maintained in serum-free medium were treated with 25 mIU/ml heparitinase 1 hr before BMP addition and throughout the remainder of the assay. Cells were then stimulated with human recombinant BMP4 at 5 ng/ml for 1 hr. Cells lysates were subjected to immunoblotting for P-Smad. Data are from triplicate cultures ± S.D (error bars) for each condition, and are normalized to total Smad. Smad phosphorylation in response to BMP4 was substantially lower in heparitinase-treated cells than in untreated cells (*; $P < 0.01$; *t*-test). (**B**) Exogenous heparin does not rescue cells from the effect of heparitinase treatment. C2C12 cells were treated with heparitinase for 1 hr, and BMP4 (5 ng/ml), or BMP4 and heparin (3-100 μg/ml) were added for a subsequent hour. Cell lysates were subjected to immunoblotting for P-Smad, and band intensities were quantified. Data are from duplicate cultures for each condition and are normalized to total Smad in cells. Smad phosphorylation in response to BMP4 was substantially lower in heparitinase-treated cells than in untreated cells (*; $P < 0.01$; *t*-test), and was not improved by addition of exogenous heparin. Heparin alone did not affect BMP4-induced Smad phosphorylation. (**C**) Dose-response curves for Smad phosphorylation in C2C12 cells (circles) and C2C12 cells treated with heparitinase (triangles). Cells were stimulated with various concentrations of BMP4 for 1 hr, lysed, and subjected to immunoblotting for phospho-Smad. The responses were reduced by about the same degree at every BMP4 concentration (asterisks denote points that are statistically significant, with $P < 0.01$; *t*-test). Data are duplicates ± S.D (error bars).

Supplemental Figure 2. Kinetic profiles of BMP2-induced p38 activation. C2C12 (**A**) or PC12 cells (**B**) were treated with heparitinase for 1 hr, and BMP2 (5 ng/ml) was added. Cell lysates were collected at indicated time points and subjected to immunoblotting for activated p38. Data are normalized to total p38 in cells.

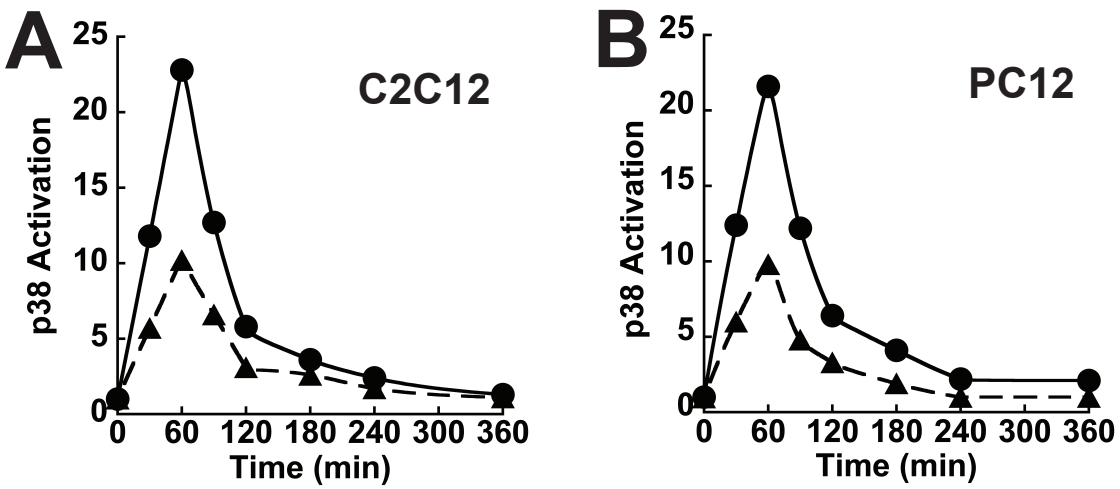
Supplemental Figure 3. Blockade of sulfation diminishes responses of cultured cells to BMP2. (A) Analysis of p38 MAPK phosphorylation in C2C12 cells. Cells were pretreated with chlorate (10 mM) for 48 hr, and BMP2 was added at 5 ng/ml for 1 hr. Cells were lysed and subjected to immunoblotting for active p38. Sorbitol (250 mM for 30 minutes) served as a positive control (PC) for p38 activation. Total p38 served as a loading control. (B) Analysis of Smad phosphorylation in C2C12 and PC12 cells. Cells were treated and samples prepared as in (A). Total Smad1/5/8 served as a loading control.

Supplemental Figure 4. Putative location of the BMP2 HS-binding domain within the hetero-oligomeric BMP-receptor complex. Onto the reported three-dimensional structure of the complex between BMP2 and the extracellular domains (ECDs) of BMPRIA and ActRII (Allendorph *et al.*, 2006), a 12-amino acid N-terminal alpha-helical extension of each BMP2 monomer has been added (dashed lines, surrounded by black oval), corresponding to one possible conformation of the BMP2 HS-binding domain (which is not resolved in published crystal structures). The view is from the plasma membrane. BMP2 monomers are shown in red, the two BMPRIA-ECDs are in blue, and the ActRII-ECDs are in green. Dashed lines represent C-terminal residues connecting the extracellular and membrane-spanning segments of the receptors. Cysteines are shown as

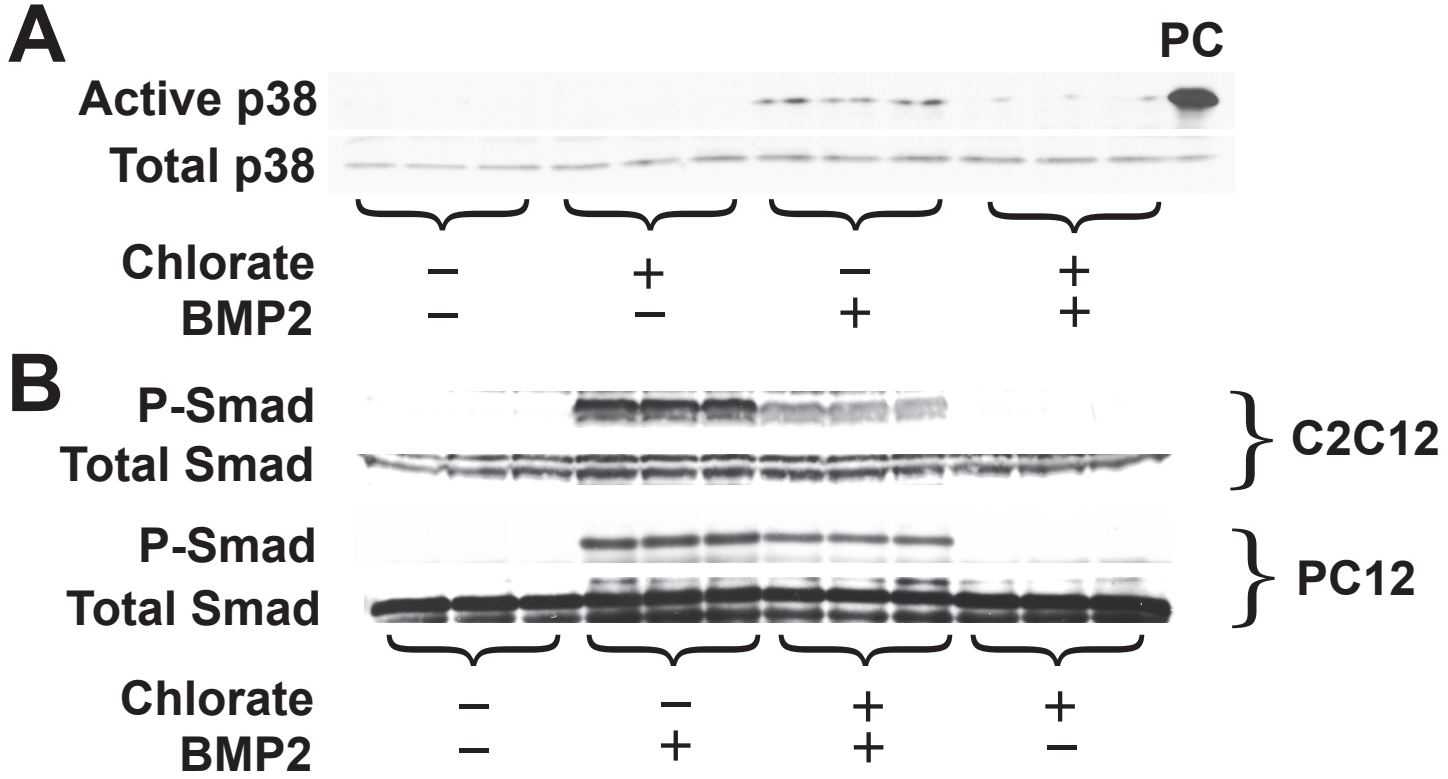
yellow spheres. Plus-signs signify the presence of 6 positive charges in each HS-binding domain. The image suggests ways in which the HS-binding domain might have an "autoinhibitory" effect on receptor assembly. For example, although the HS-binding domain is located relatively far from the interface between BMP2 and the type II receptor subunit (and so is unlikely to simply sterically hinder ligand-type II receptor interaction), it is close to the strongly anionic L23 loop (4 of 6 residues are aspartate or glutamate) of the type I receptor units, as well as to the plasma membrane. Thus the HS-binding domain might engage in electrostatic interactions with the L23 loop of the type I receptor subunit, or with anionic moieties on extracellular face of the plasma membrane.



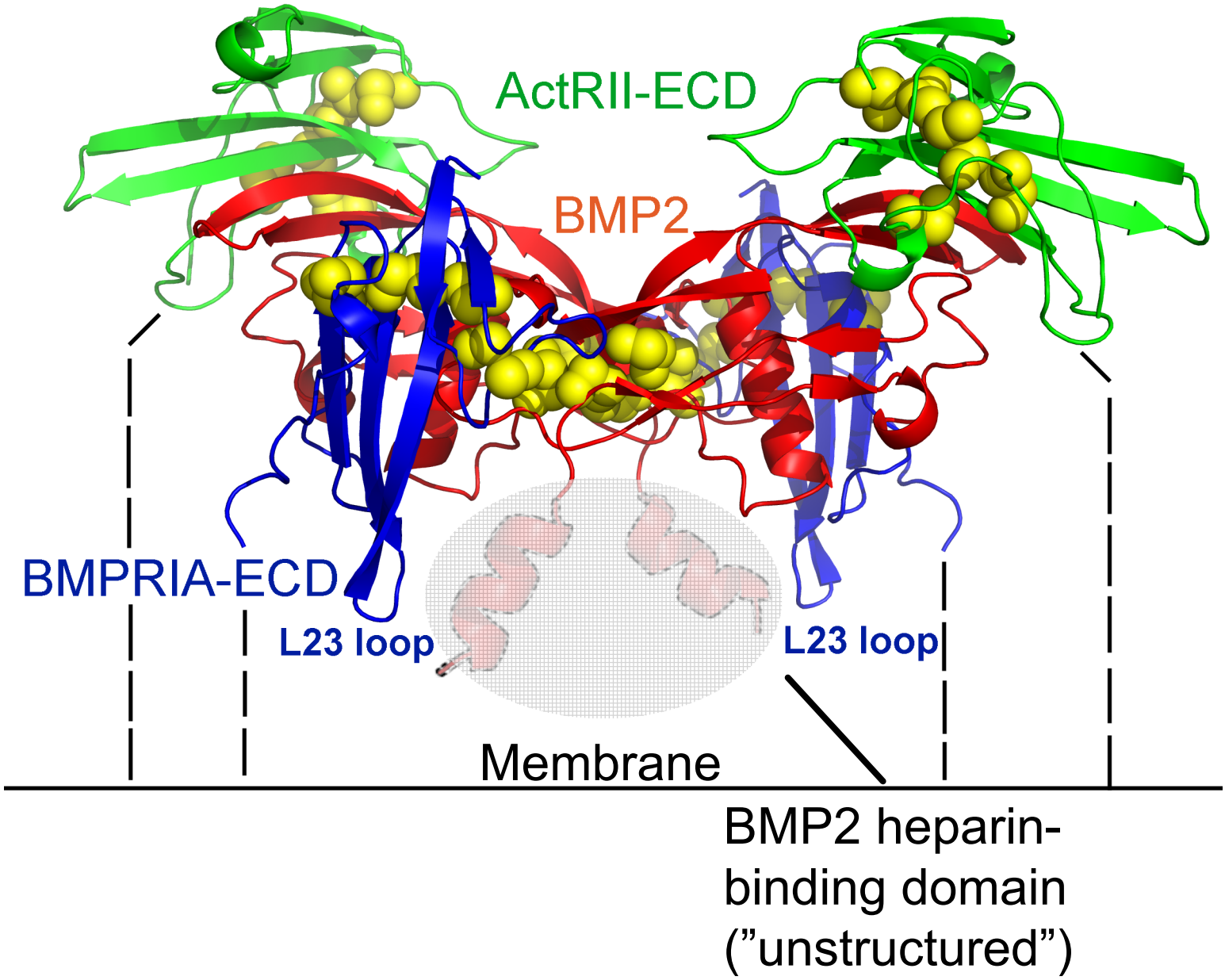
Supplemental Fig. 1



Supplemental Fig. 2



Supplemental Fig. 3



Supplemental Fig. 4