SUPPLEMENTARY FIGURE LEGENDS

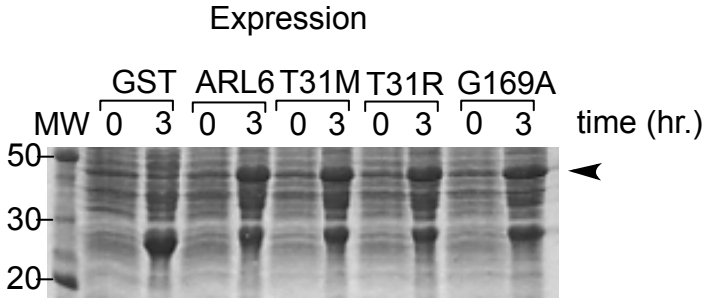
Supplementary Figure 1. Biochemical data corroborates structural predictions on the nucleotide binding capabilities of GST-tagged pathogenic BBS3 variants. (A) Coomassie-stained SDS gel showing induction of GST, wild-type GST-ARL6, and the GST-T31M, GST-T31R, and GST-G169A mutants. Arrowheads indicate the position of the GST fusion proteins. Molecular weight (MW) is indicated in kDa. (B) Coomassie-stained SDS gel showing proportion of total cell lysate (T) from the cells in (A) that remains in the soluble fraction (S). (C) An aliquot of the soluble fraction from (B) was spotted onto nitrocellulose membrane and probed with α-^{32}P-GTP. (D) An aliquot of the soluble fraction from (B) was spotted onto nitrocellulose membrane and probed with γ-^{32}P-GTP.

Supplementary Figure 2. Western blot showing specificity and sensitivity of ARL6C and ARL6N antibodies. (A) Western Blot analyses with ARL6C antibody of recombinant *H. sapiens* ARF6 and ARL6/BBS3 (left panel) loaded side-by-side to show antibody specificity, and (right panel) dilution series of purified recombinant ARL6 showing antibody sensitivity. (B) As with (A), except that the Western Blot analysis was performed with the ARL6N antibody.

Supplementary Figure 3. Immunolocalisation of ARL6/BBS3 to basal bodies in different mammalian tissue sections. Staining for ARL6, using the ARL6C antibody (green), and for basal bodies using a γ–tubulin antibody (red) shows ARL6 localising to basal bodies in (A) mouse testis and in (B) the mouse brain. (C) In the kidney, ARL6 (green) colocalises with the centrosomal/basal body marker M4491 (red).

Supplementary Figure 4. Overexpression of wild-type but not mutant forms of ARL6/BBS3 alters responsiveness to Wnt3a ligand. In 293T TOPflash reporter cells, overexpression of ARL6/BBS3 leads to increased β-catenin activity in response to Wnt3a ligand, but no global dysregulation of β-catenin signalling. In contrast, overexpression of (A) pCMV-BBS3(T31R), pCMV-BBS3(Q73L), (B) pcDNA6.2-GFP-BBS3(G169A) and pcDNA6.2-GFP-BBS3(L170W) has no marked effect on β-catenin transcriptional activity. Cells are consistently 70-80% confluent and ciliated as observed by staining with an anti-acetylated tubulin antibody (not shown). Error bars represent standard deviation; for (A) and (B), only the BBS3 WT samples are statistically different from the empty vector control ($p < 0.05$).

A



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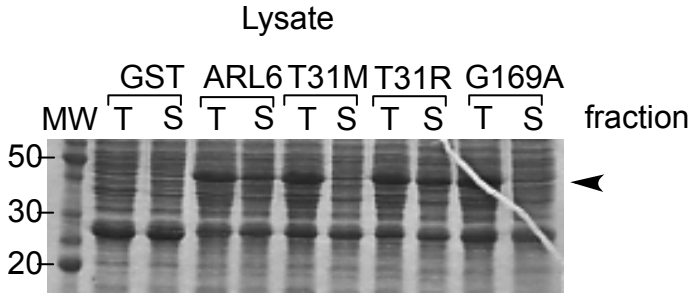

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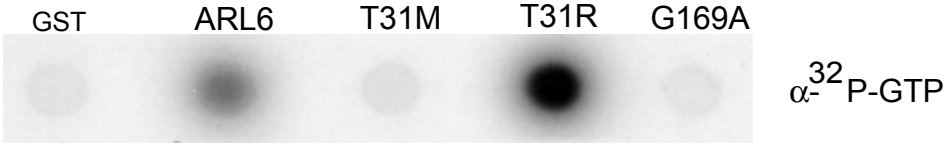

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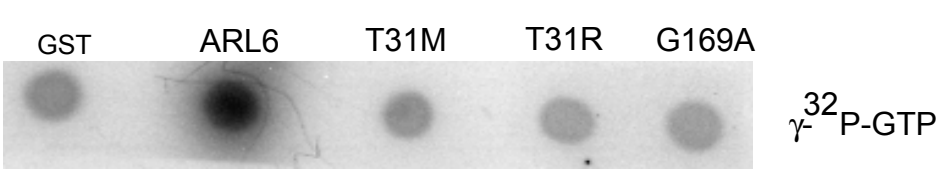
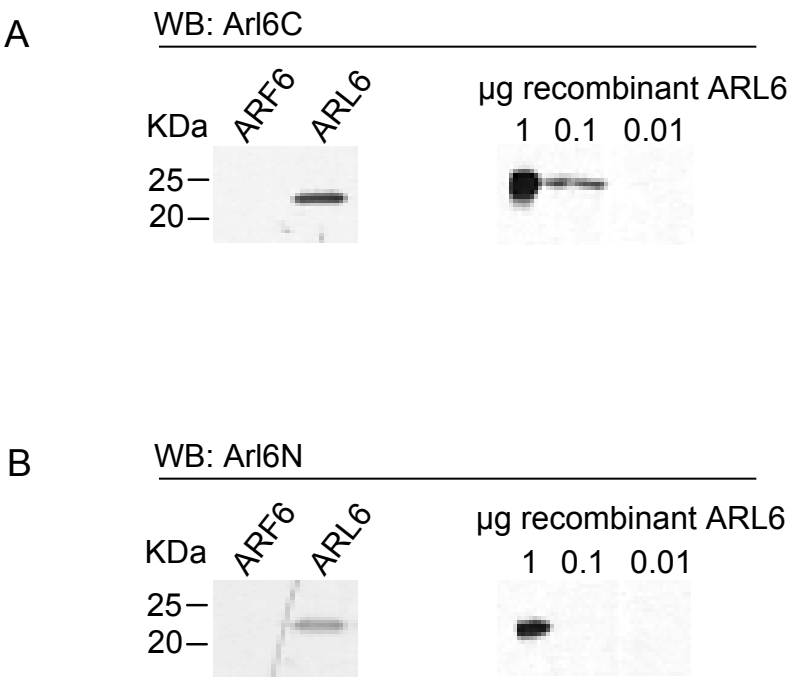
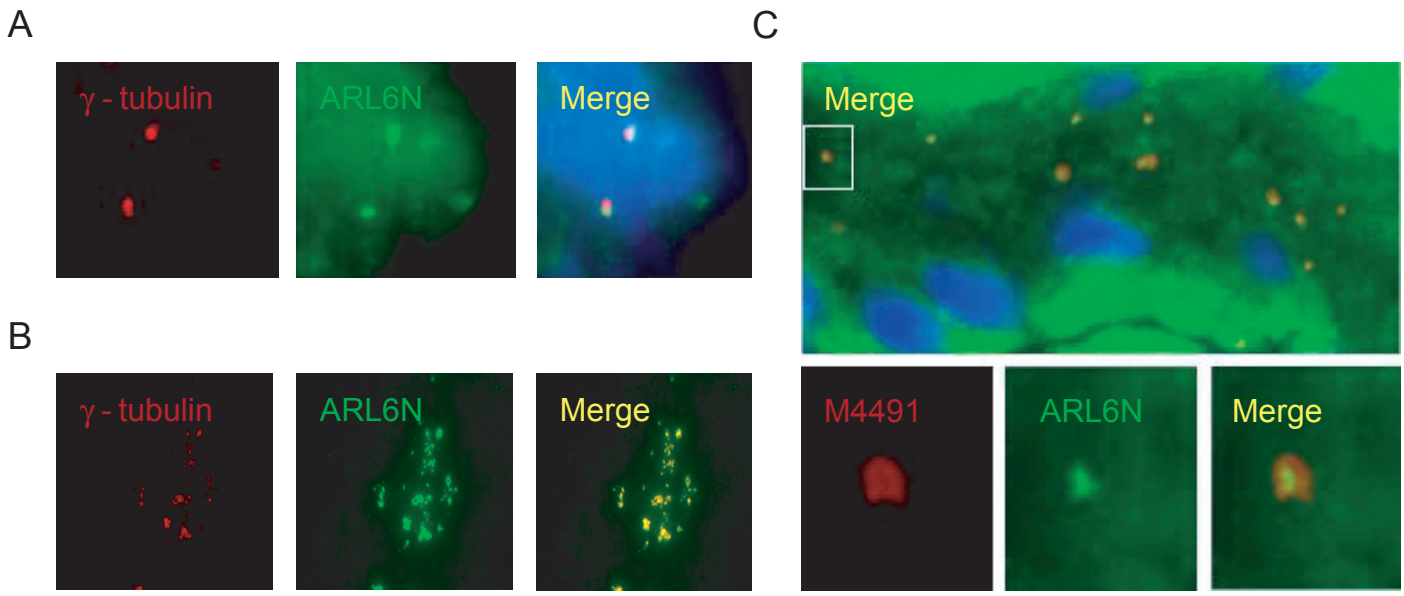


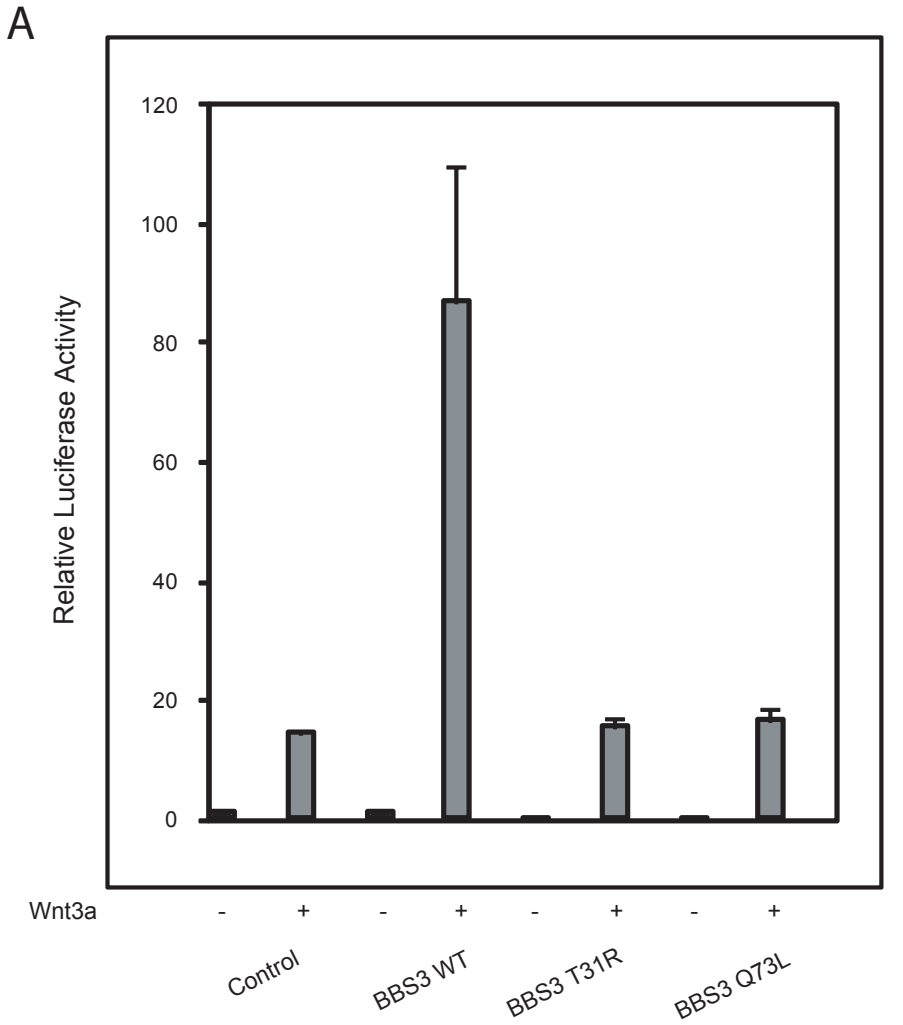
Wiens et al., Supplementary Figure 1

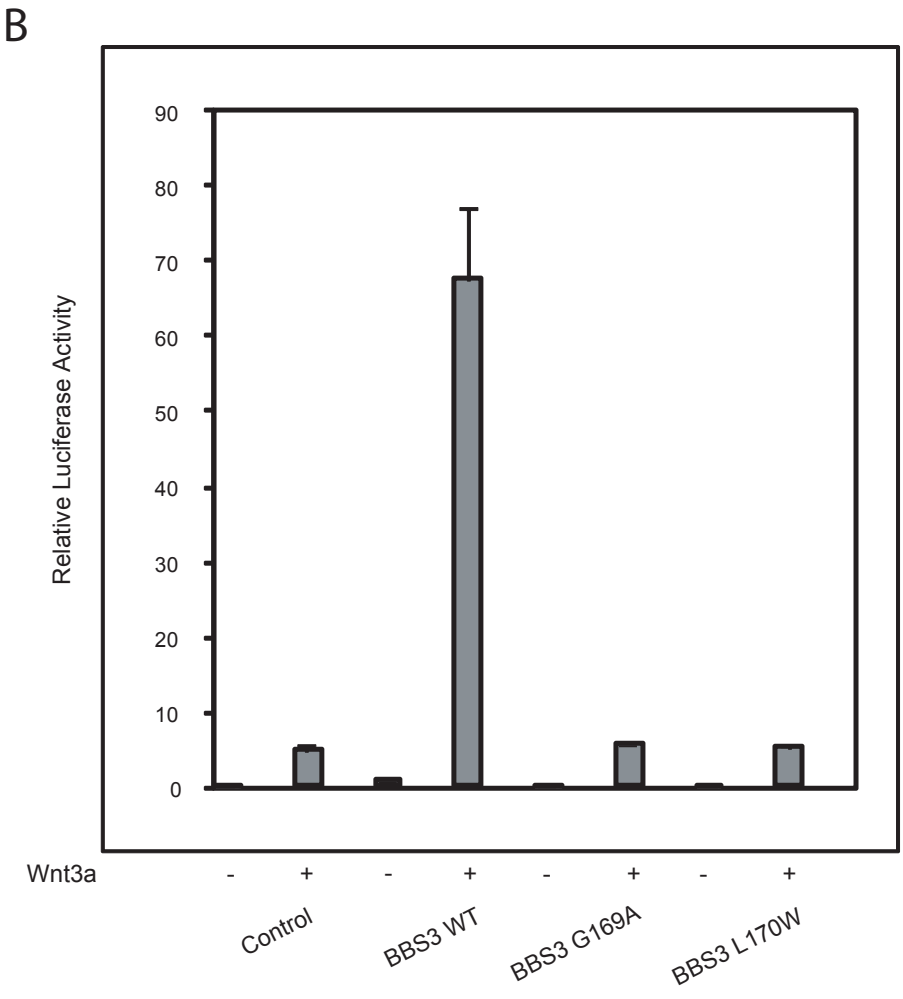


Wiens et al., Supplementary Figure 2



Wiens et al., Supplementary Figure 3





Wiens et al., Supplementary Figure 4