

Supplementary Information

Serum albumin maintains Wnt water-solubility and activity

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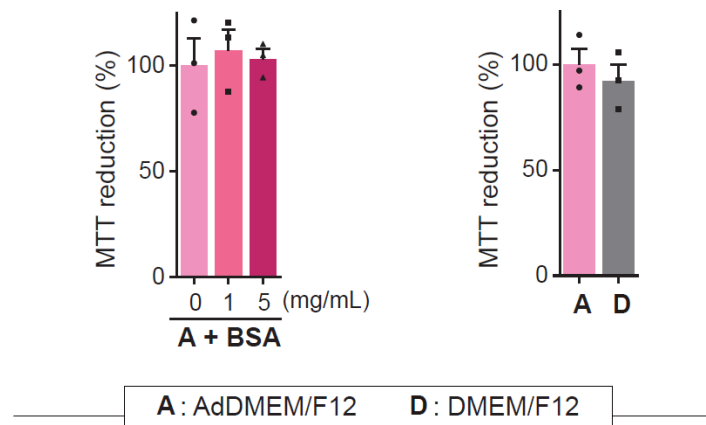
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This Supplementary Information PDF contains the following:

Supplementary Figures 1-4

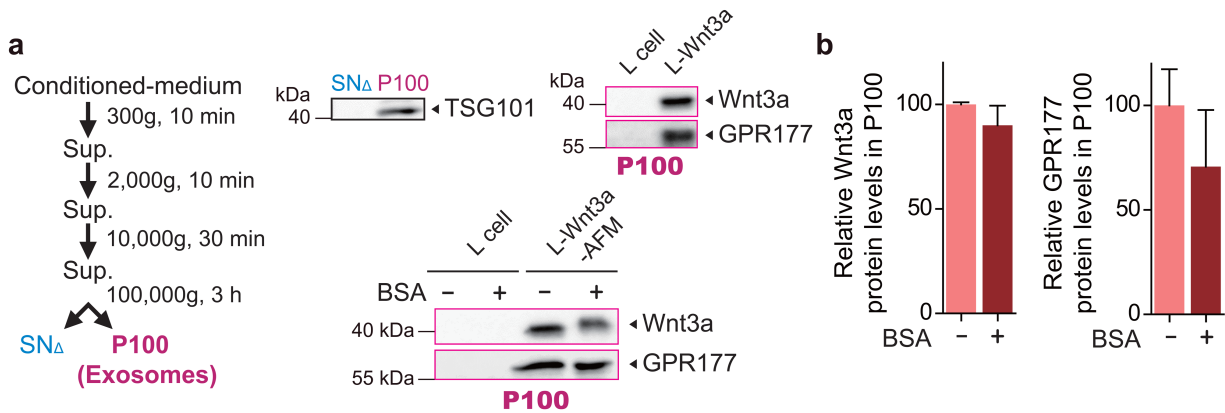
Supplementary Table 1

Uncropped blots of western blotting analysis



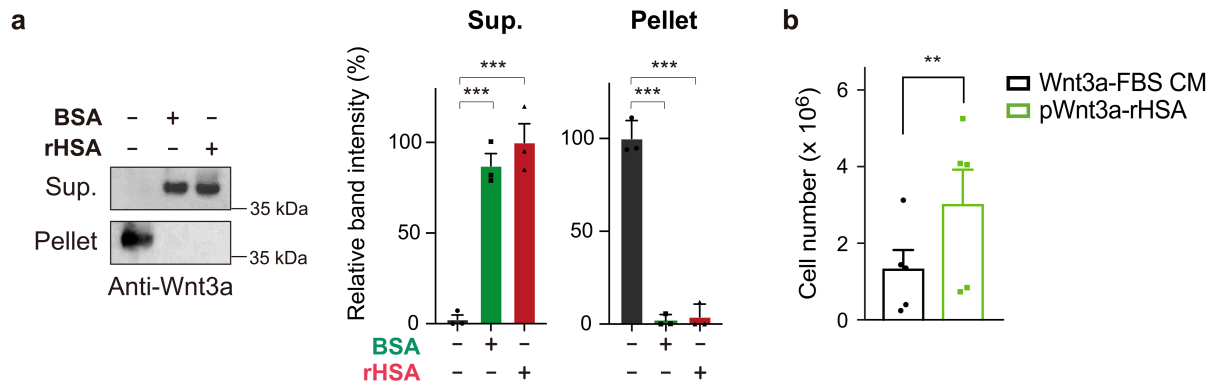
Supplementary Figure 1. MTT assays in L-Wnt3a-AFM cells, Related to figure 1

L-Wnt3a-AFM cells were cultured in the indicated media for 5 days and then MTT assays were performed to measure cell viability or cell proliferation. (Left) BSA (1 or 5 mg/mL) was added to AdDMEM/F12 for 5 days. (Right) L-Wnt3a-AFM cells were cultured in AdDMEM/F12 or DMEM/F12.



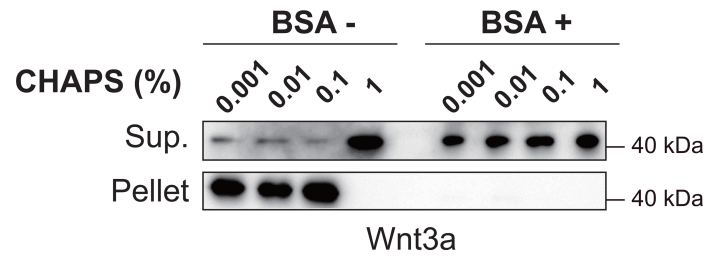
Supplementary Figure 2. BSA does not affect Wnt3a secretion on exosomes, Related to figure 3

(a) Exosomes were purified from CM produced in cultures of L-Wnt3a-AFM, L-Wnt3a, or L cells (as a negative control) with or without BSA (5 mg/mL). Differential centrifugation of CM separated it into SN_Δ fractions and P100 exosome fraction. Western blotting for the exosome marker TSG101 confirmed that P100 was enriched with exosomes. Wnt3a was present in P100 pellets of L-Wnt3a or L-Wnt3a-AFM CM (based on DMEM/F12) along with its cargo protein GPR177 (Wntless). BSA did not affect the amount of Wnt3a and GPR177 in exosomes. (b) A quantification of Wnt3a and GPR177 in exosomes is depicted in bar graphs.



Supplementary Figure 3. rHSA and BSA show similar effects, Related to figure 5 and 6

(a) Purified Wnt3a (200 $\mu\text{g/mL}$ with 0.5% CHAPS) was added to a final concentration of 500 ng/mL in DMEM/F12 containing vehicle, BSA (5 mg/mL), or rHSA (5 mg/mL). Then, the partitioning of Wnt3a into soluble or insoluble fractions was examined via anti-Wnt3a immunoblotting (left). Relative Wnt3a protein levels in supernatants and pellets are depicted in bar graphs (right). Data ($n = 3$) are presented as means $\pm$ SEM. *** $P < 0.001$ compared to BSA-, rHSA- control. (b) Growth rate of human colon organoids in indicated media (Wnt3a-FBS CM or pWnt3a-rHSA). Cell numbers of each well were counted and the cumulative number of cells after passage 2 was calculated by the equation mentioned in the Method. ** $P < 0.01$, ratio paired t test compared to Wnt3a-FBS CM. Data ($n = 5$) are presented as means $\pm$ SEM.



Supplementary Figure 4. BSA efficiently solubilizes Wnt3a even in trace amounts of CHAPS, Related to figure 5

Solubility assays were performed with purified Wnt3a co-incubated with different concentrations of CHAPS in the presence or absence of BSA (5 mg/mL). Supernatants (Sup.) and pellets were subjected to western blotting using anti-Wnt3a antibodies.

Components	C _{DMEM/F12} (mg/L)	C _{Advanced DMEM/F12} (mg/L)
AlbuMAX® II	-	400
Insulin Recombinant Full Chain	-	10
Human Transferrin (Holo)	-	7.5
Ascorbic Acid phosphate	-	2.5
Glutathione, monosodium	-	1
Sodium Selenite	-	0.005
Ammonium Metavanadate	-	0.0003
Manganous Chloride	-	0.00005
L-Glutamine	365	- *

* The same amount of L-glutamine was added when used in experiments

Supplementary Table 1. Formulation comparison between DMEM/F12 and advanced DMEM/F12, Related to figure 1.

The identical components in both media are not presented.

Uncropped blots of western blotting analysis

Figure 1F

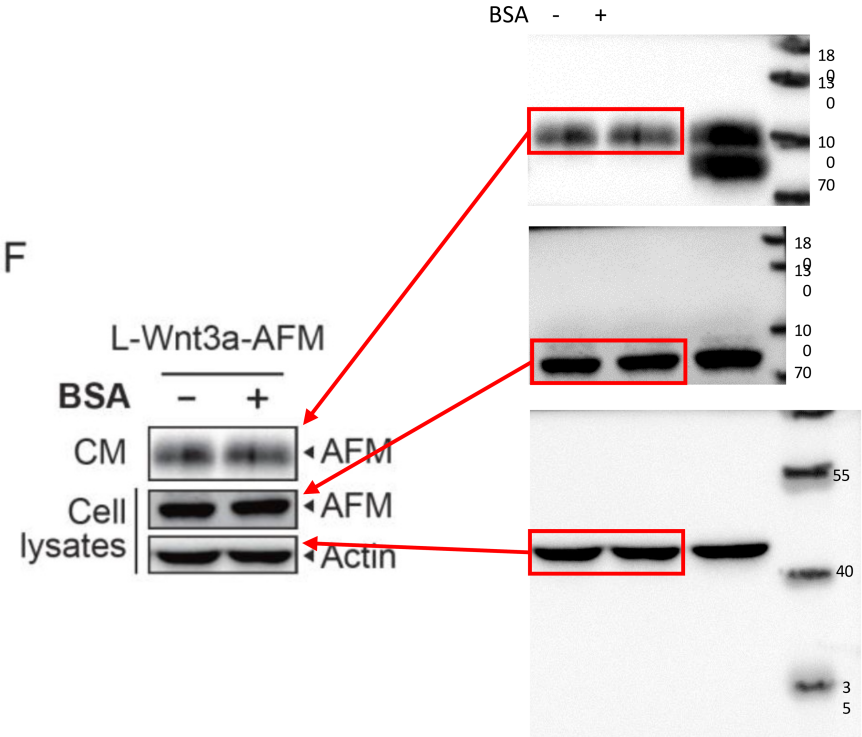


Figure 3A

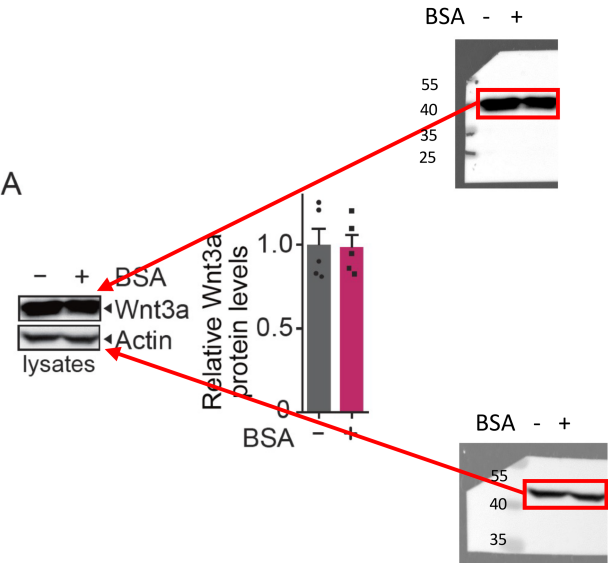


Figure 3B

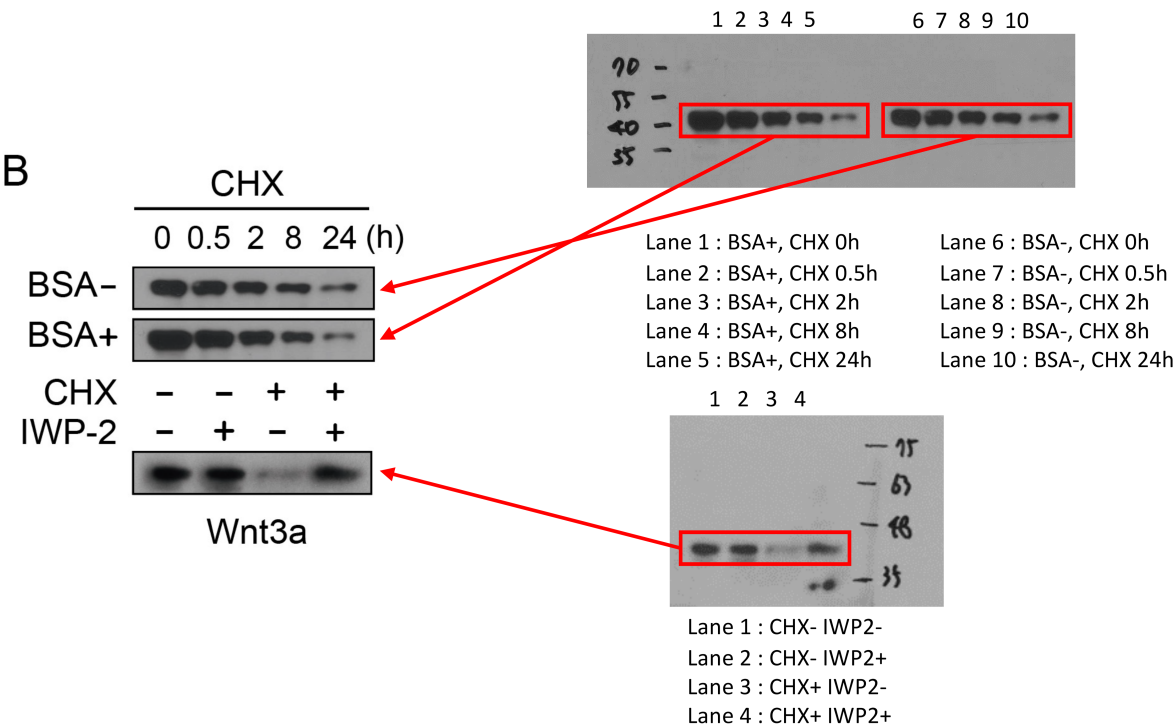


Figure 3C

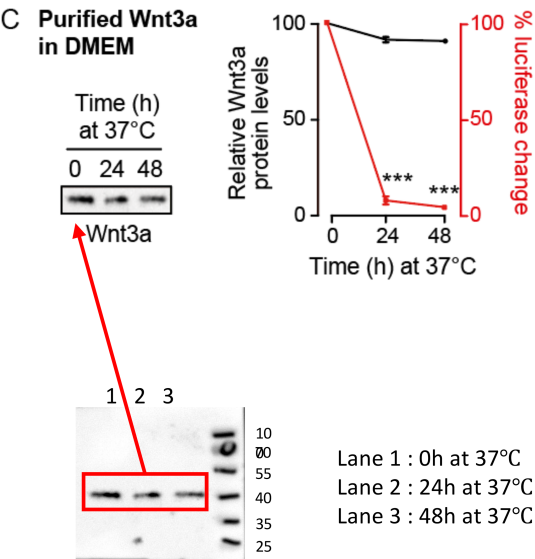


Figure 3D

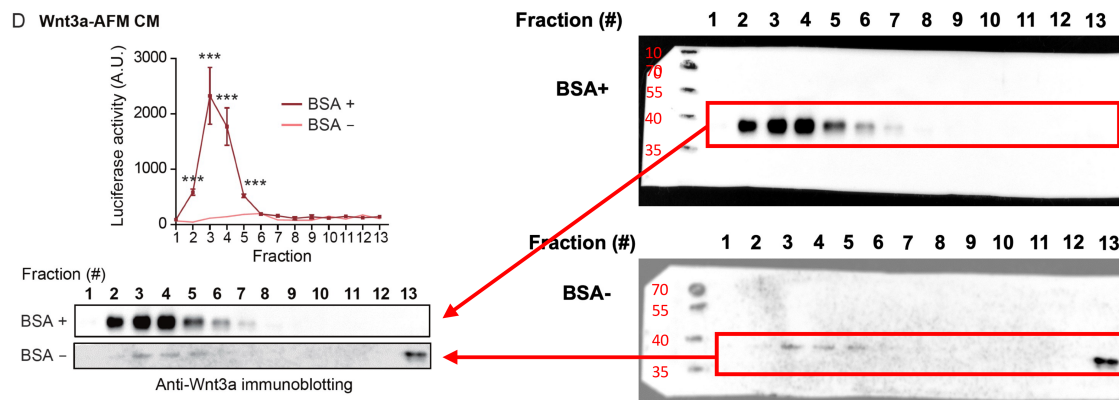


Figure 3E

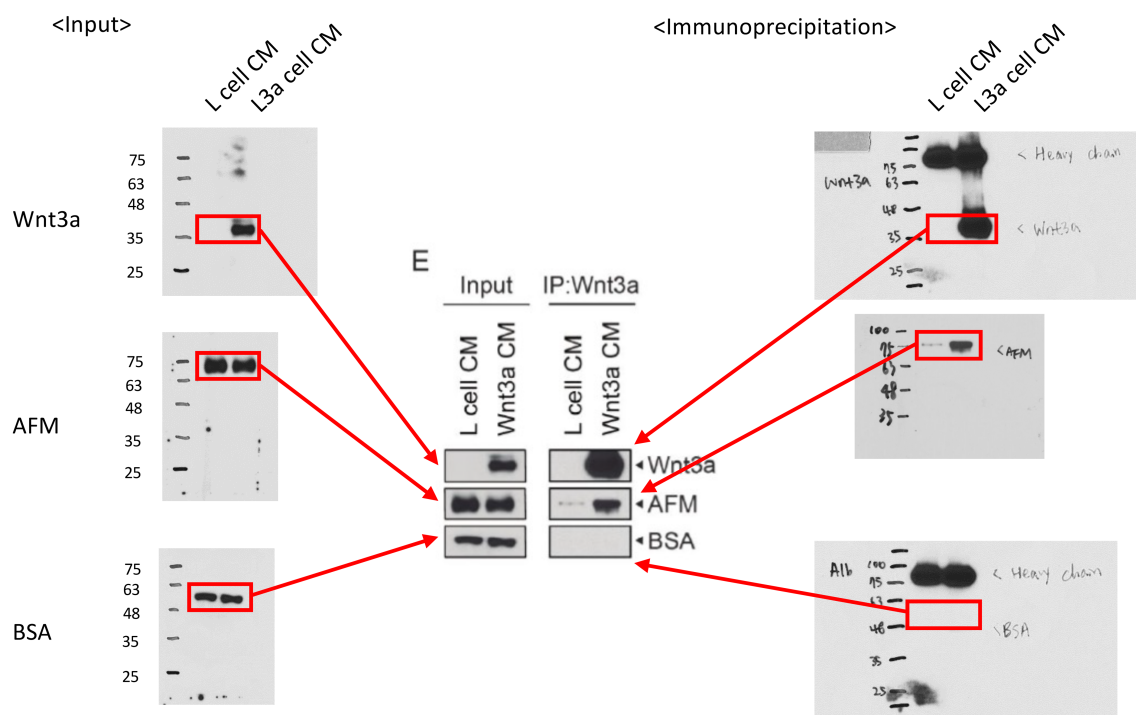


Figure 5B

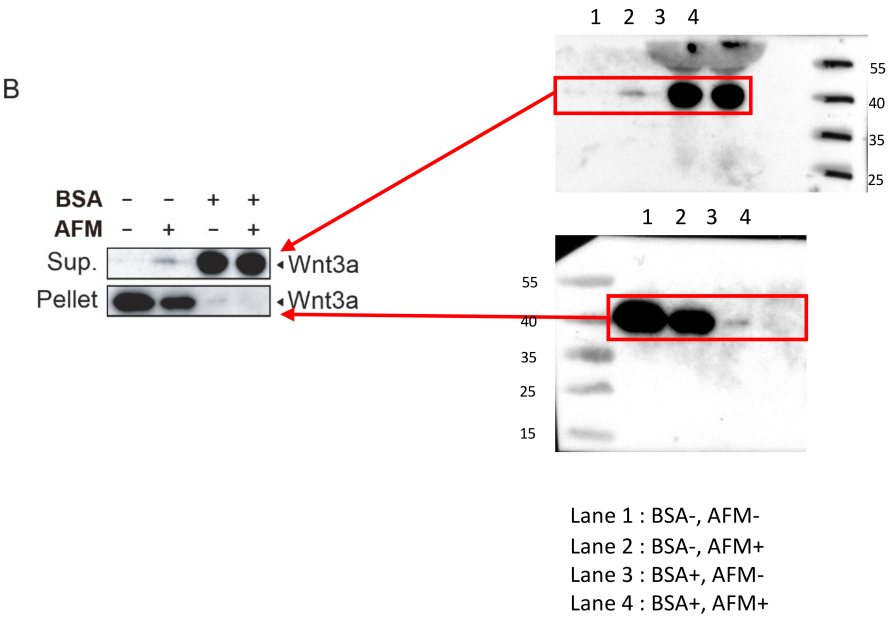


Figure 5C

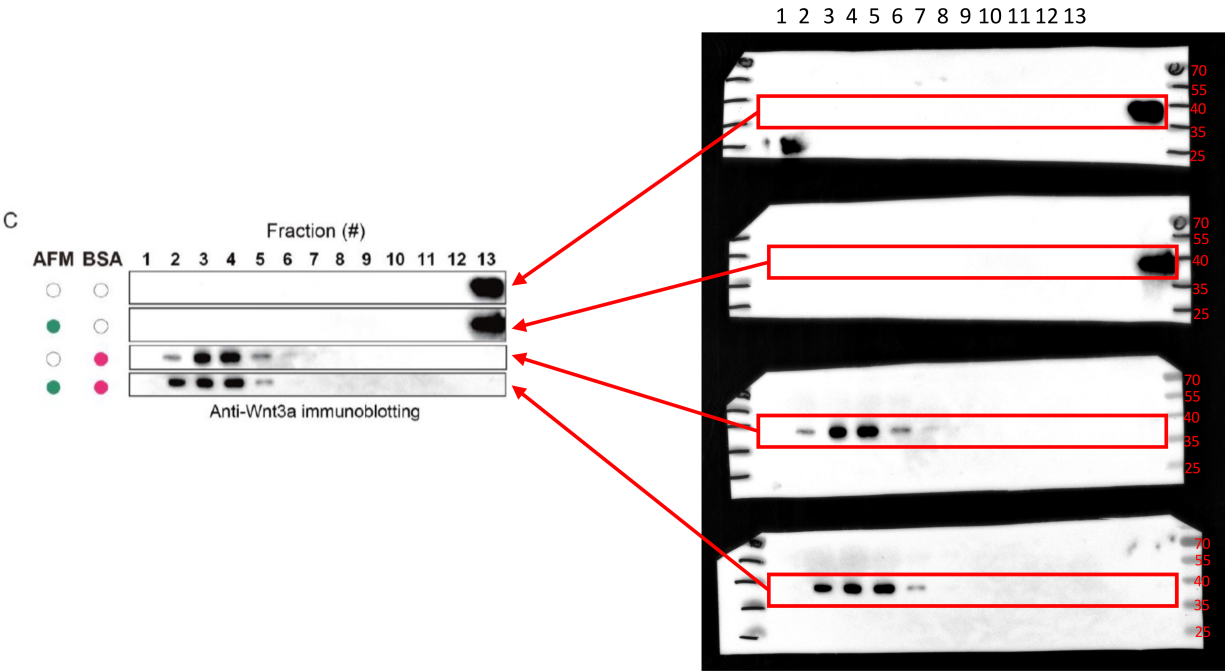


Figure 5D

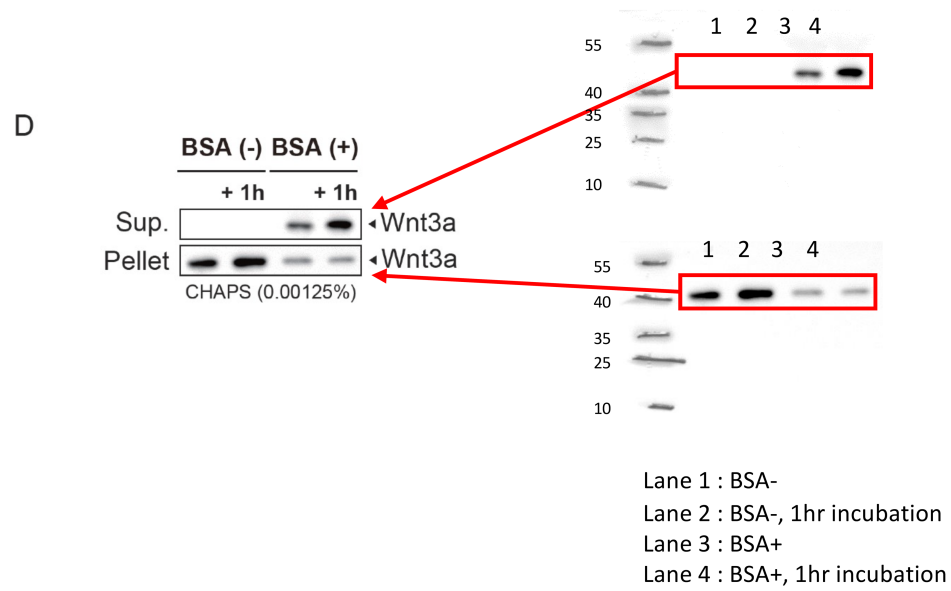


Figure 5E

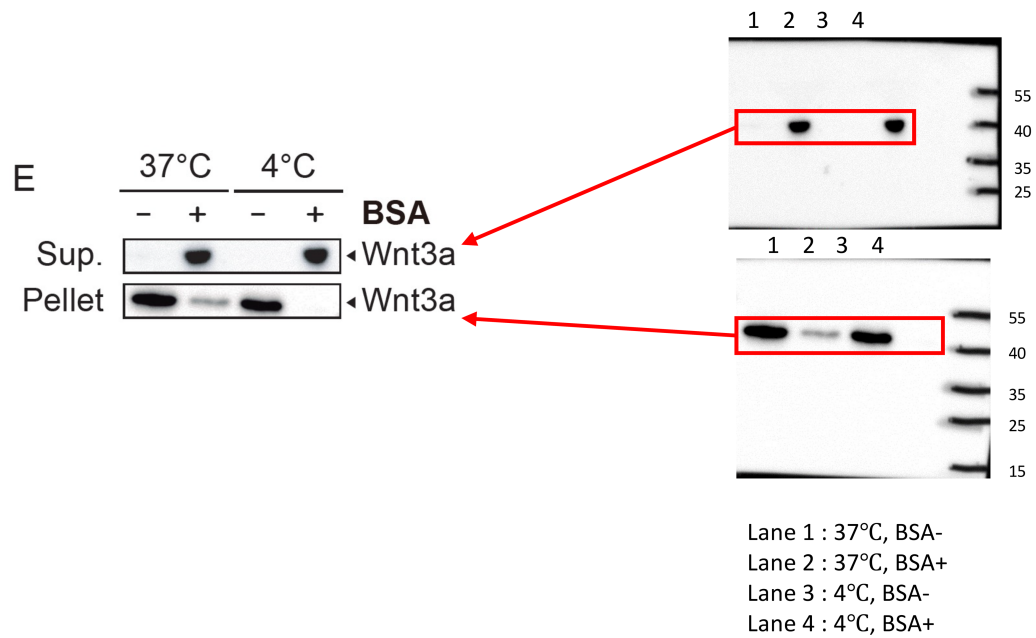


Figure 5F

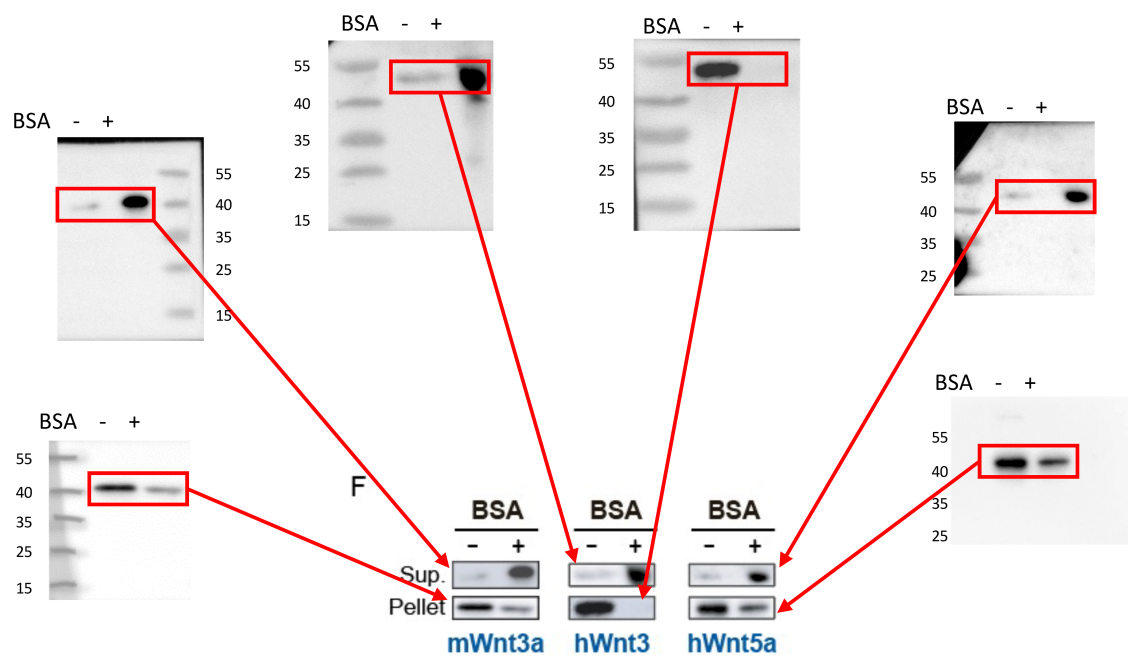


Figure 6A

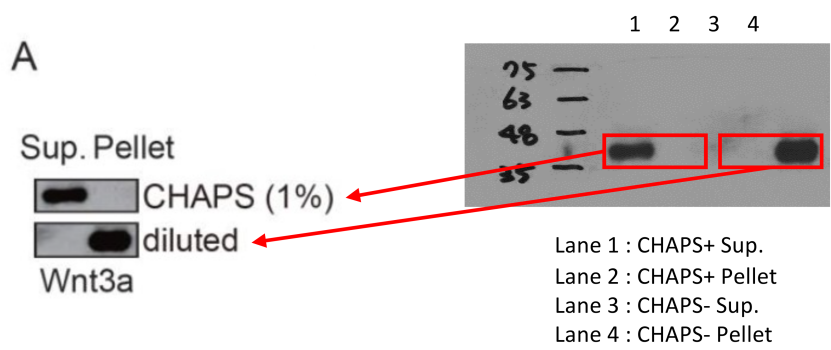


Figure 5F

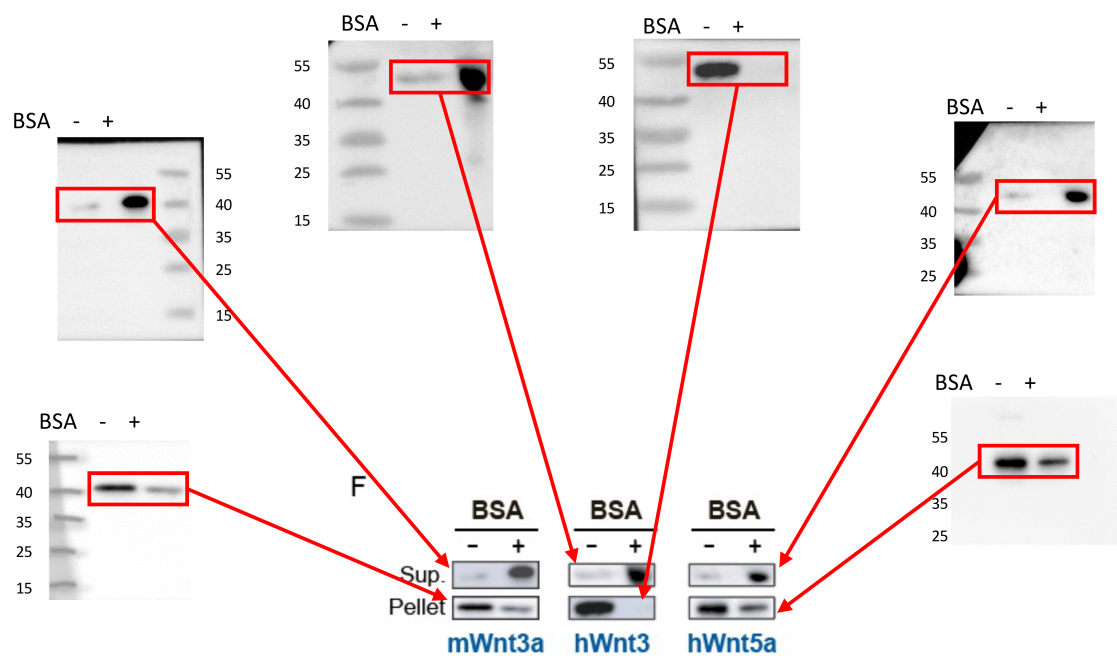


Figure 6A

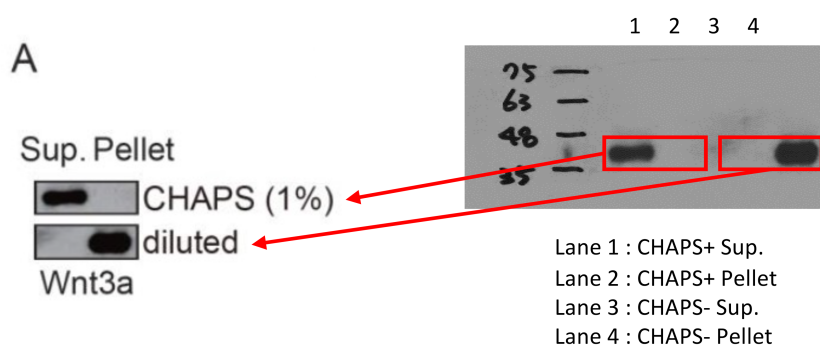


Figure 6C

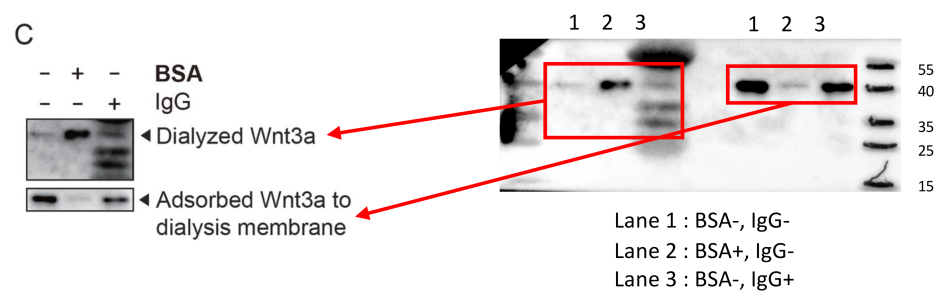


Figure 6D

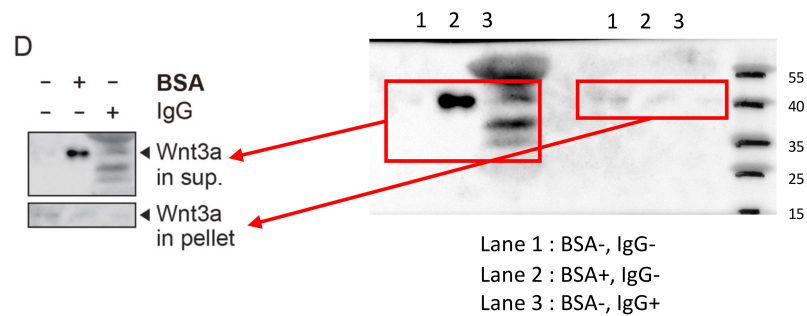


Figure 6E

