SUPPLEMENTARY INFORMATION LEGENDS

Figure S1. Time course of GSK-3β stimulation by Wnt ligands (100 ng/ml). Changes in the intracellular localization of PKCζ and GSK-3β occur in a short time (5 min) while after 15 min, this returns to baseline in both non-malignant 112CoN cells (panel A) and malignant RKO and SW480 cells (panels B and C, respectively). Cells were fixed, permeabilized and co-immunostained with antibodies against GSK-3β and against PKCζ. Fluorescence was analyzed by laser confocal microscopy as described in the Experimental Procedures section. PKCζ was visualized with FITC-conjugated goat anti-rabbit antibody and GSK-3β with rhodamine goat anti-mouse antibody. Data are representative of three independent experiments.

Figure S2. Efficiency of the knockdown of PKCζ. RKO or SW480 cells were transfected with 2 μg of pSuperPKCζ-RNAi plasmid or with control pSuper plasmid using Lipofectamine 2000. The efficiency of the silencing of PKCζ was determined 36 h post-transfection of cells by Western blot.

Figure S3. I*n silico* analysis of the GSK-3β aminoacid sequence showing the putative PKC phosphorylation sites in GSK-3β. The sites obtained from the analysis appear underlined in black; three sites displaying the classical PKC consensus sequence with high probability score are underlined in red, and the Serine 147, that exactly matched with the

consensus sequence recognized by the P-Ser-PKC-substrate antibody used in the experiments ($R/K_{(0-2)}$ Ser X $_{(hydrophobic)}R/K$) is shown underlined with a curly red line.

Figure S4. Efficiency of transfection of GSK-3β wild-type, GSK-3β/S147A and GSK-3β/S147E. RKO cells were transfected with the void plasmid (vector) or with plasmid-encoding wild type GSK-3β or plasmid–encoding GSK-3β mutants. GSK-3β was immunoprecipitated 24 h post-transfection from cell extracts and analyzed by Western blot. Actin antibody was used to control for equal loading. The results are representative of three independent experiments using different cell preparations.

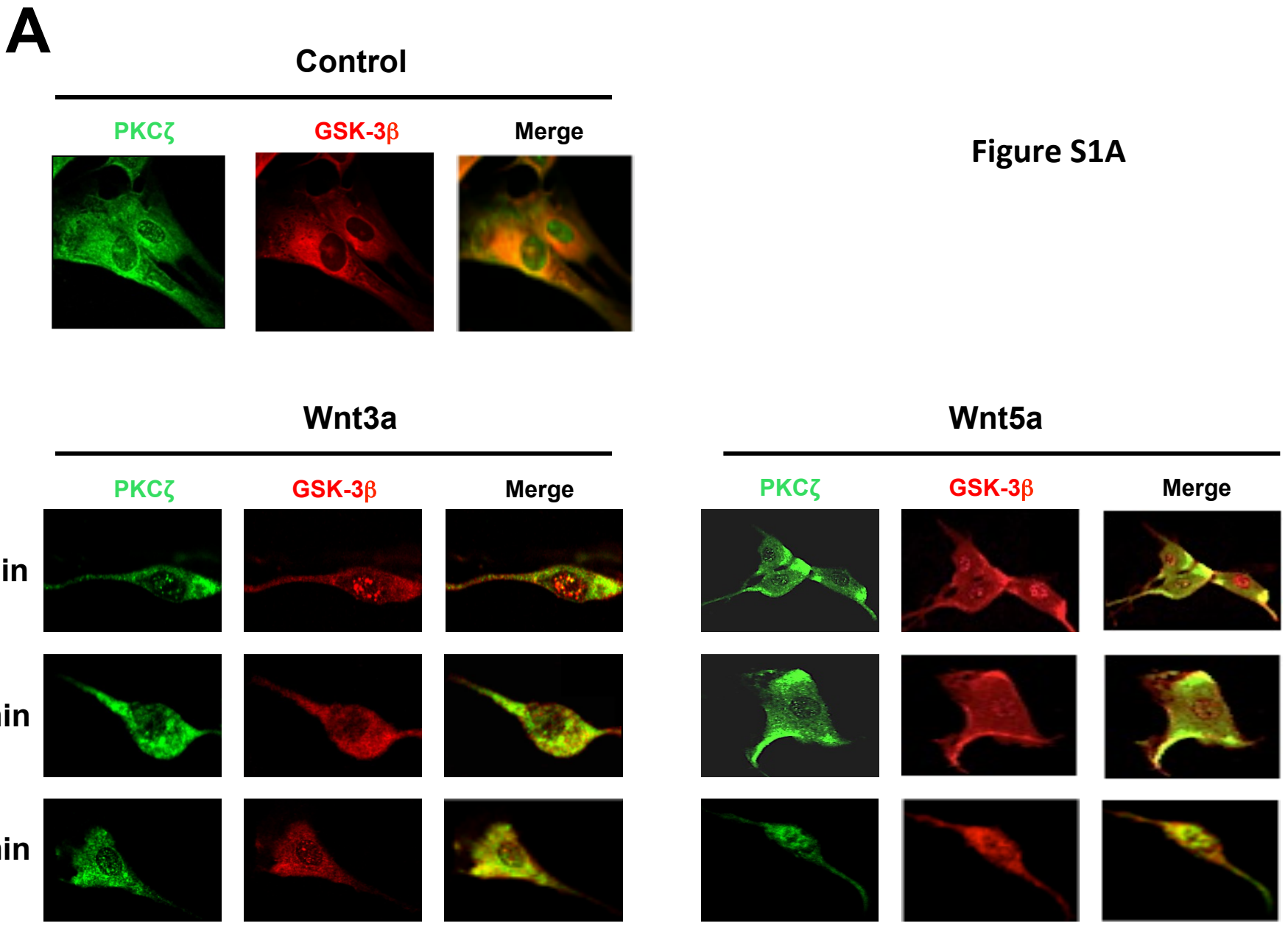
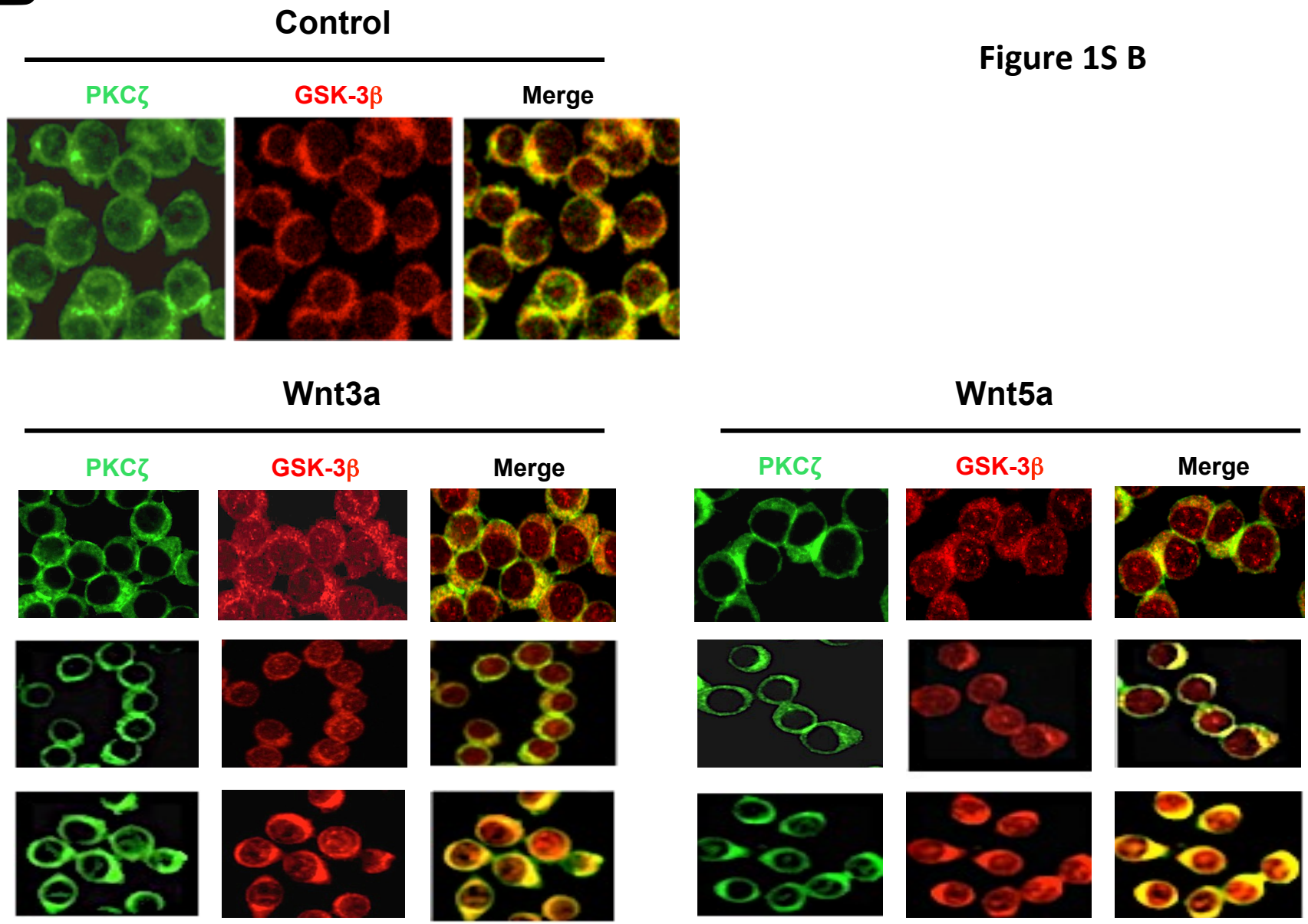

A
Control
PKCζ
GSK-3β
Merge
Wnt3a
PKCζ
GSK-3β
Merge
Wnt5a
PKCζ
GSK-3β
Merge
5 min
15 min
30 min

Figure S1A

B

Figure 1S B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Merge
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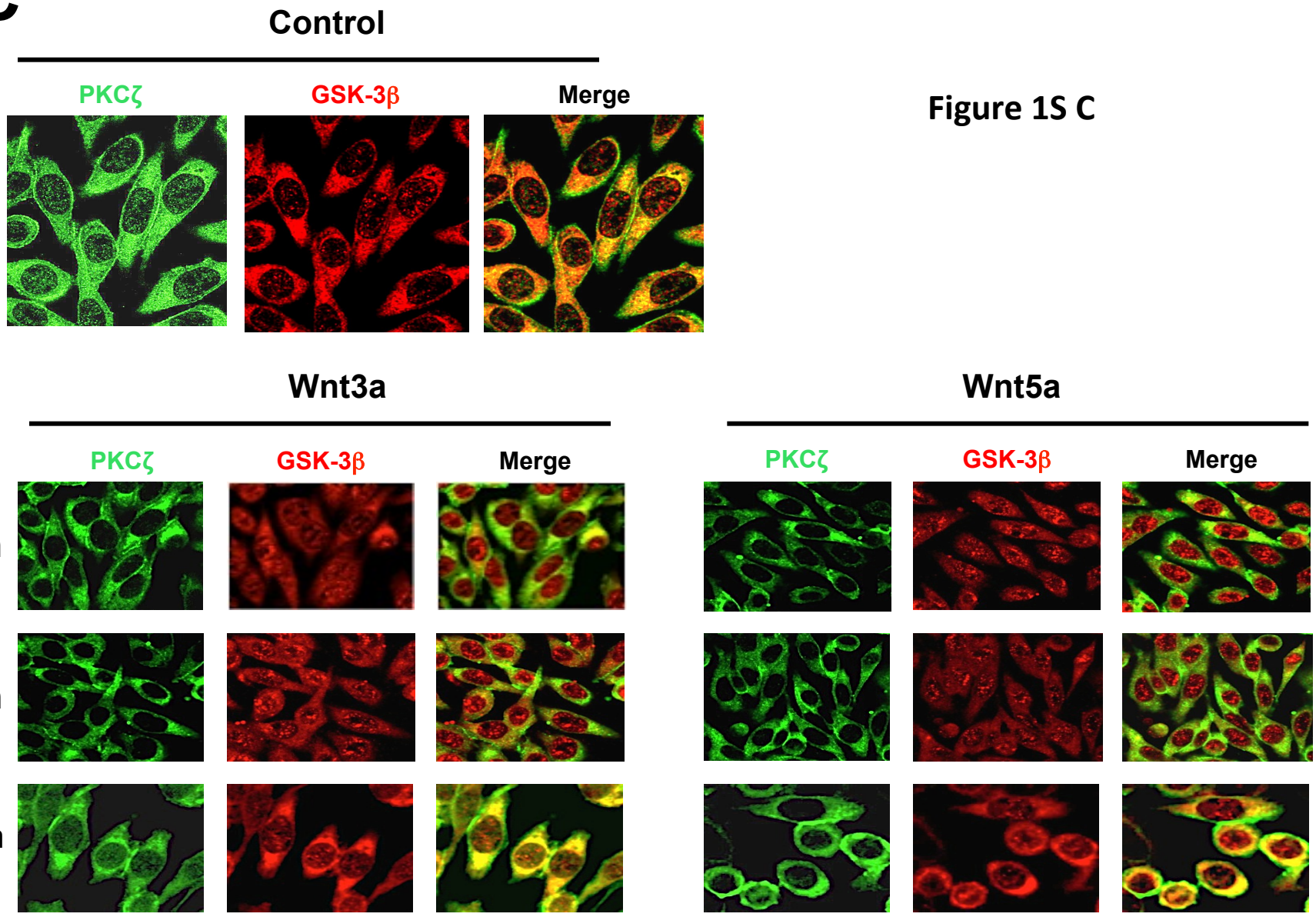
GSK-3β
Merge
5 min
15 min
30 min

C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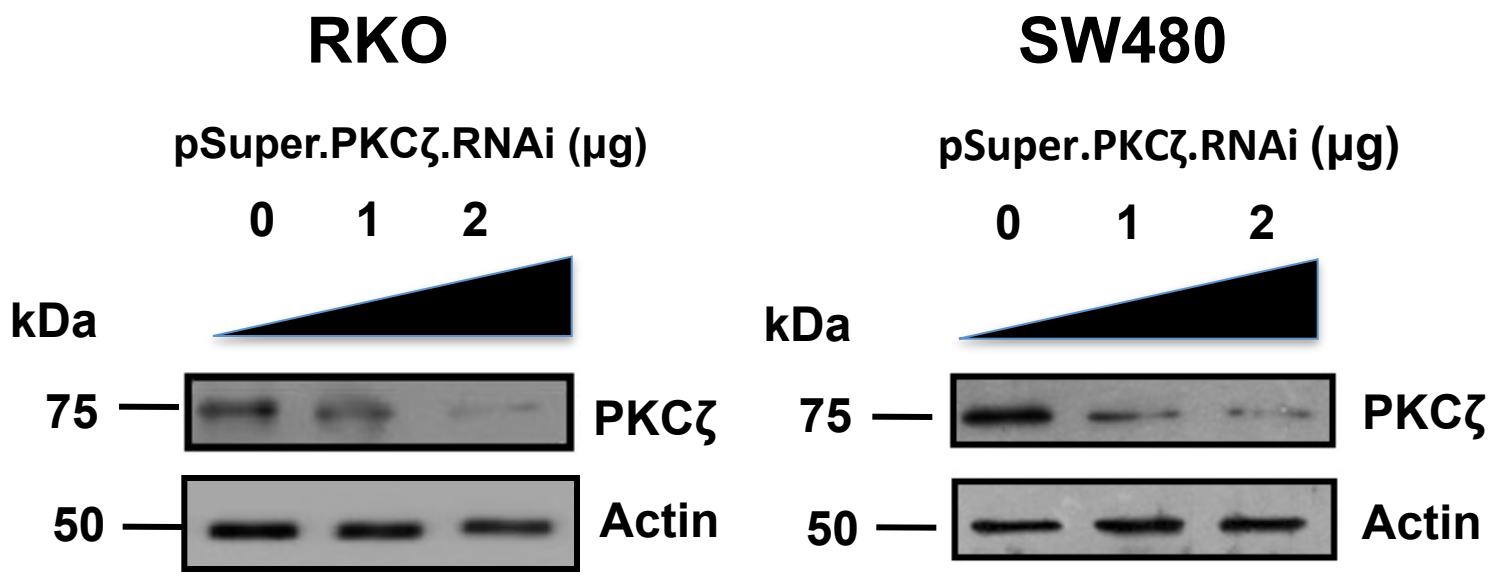
GSK-3β
Merge
5 min
15 min
30 min

Figure 1S C



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>gi|21361340|ref|NP_002084.2| glycogen synthase kinase-3 beta isoform 1 [Homo sapiens]

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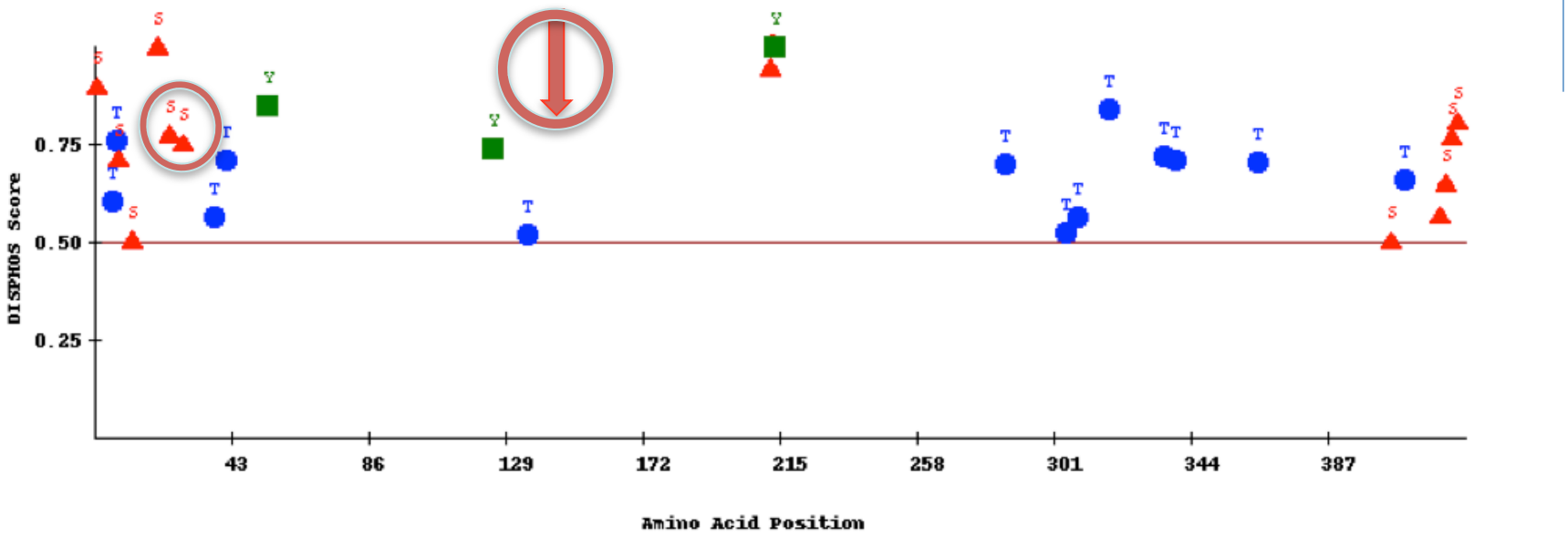
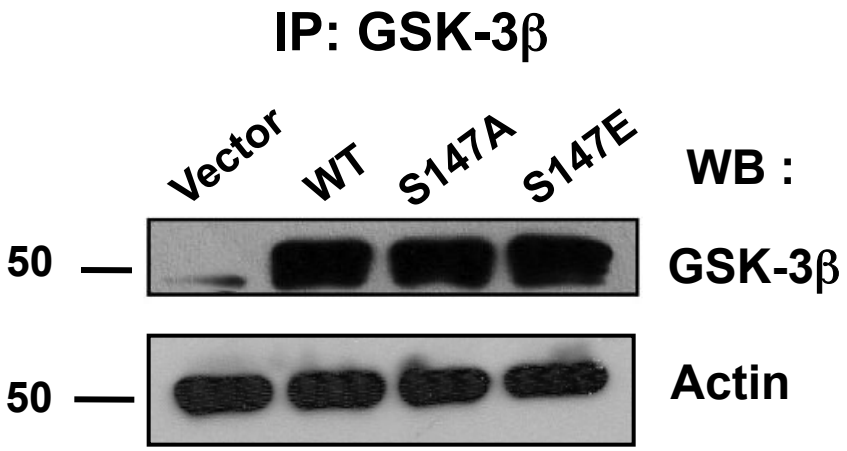


http://www.dabi.temple.edu/disphos/pred/predict

Figure S3. Putative PKC phosphorylation sites in GSK-3β (NetphosK 2.0) The sites obtained from the analysis appear underlined in black; three sites displaying the classical PKC consensus sequence with high probability score are underlined in red, and the Serine 147, that exactly matched with the consensus sequence recognized by the P-Ser-PKC-substrate antibody used in the experiments ($R/K_{(0-2)}$ Ser X $_{(hydrophobic)}R/K$) is shown underlined with a curly red line.



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