Rac1 activation upon Wnt stimulation requires Rac1 and Vav2 binding to p120-catenin.

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Supplementary Figure Legends

Suppl. Figure 1. Wnt-induced JNK2 phosphorylation is prevented by p120-catenin depletion. (A) SW-480 stably expressing scrambled or shRNA specific for p120-catenin were treated with Wnt3a-conditioned media for 2 hours and lysed. Phospho-JNK2 and total JNK2 were detected from extracts by WB. (B) Autoradiograms from five different experiments as in (A) performed in duplicate were quantified and the mean ± SD was obtained for each condition. Each value is presented relative to that obtained in non-depleted cells treated with control medium.

Suppl. Figure 2. Active Rac1 is required for β-catenin nuclear translocation and acts down-stream p120-catenin. (A) Dominant negative Rac1 (Rac1-N17) or (B) constitutively active Rac1 (Rac1-V12) or the empty vector were overexpressed in HEK-293 cells and SW-480 cells, respectively, and treated with control or Wnt3a-conditioned medium for 15h. Nuclear fraction was separated from the cytosolic and membrane-associated fraction as detailed in Materials and Methods and β-catenin levels in each cell compartment were analyzed by WB. Lamin-β1 was used as a nuclear marker; pyruvate kinase was used as a marker for the cytosolic-plus-membrane fraction.

Suppl Figure 3. p120-catenin is released from E-cadherin after Wnt stimulation. E-cadherin was immunoprecipitated from 500 μg of SW-480 whole-cell extracts treated with control or Wnt3a-

conditioned medium for 2 hours. 4 μg of SW-480 whole-cell extracts were included as input. Protein complexes were analyzed by WB.

Suppl. Figure 4. Wnt-induced JNK2 phosphorylation is dependent on Vav2. (**A**) SW-480 stably expressing scrambled or shRNA specific for Vav2 were treated with Wnt3a-conditioned media for 2 hours and lysed. Phospho-JNK2 and total JNK2 were detected by WB. (B) Autoradiograms from five different experiments as in (A) performed in duplicate were quantified and the mean ± SD was obtained for each condition. Each value is presented relative to that obtained in non-depleted cells treated with control medium.

Suppl. Figure 5. Rac1 directly interacts with p120-catenin. (A) Diagram of the different domains of p120-catenin. The length of the p120-catenin deletion mutants used in this work is shown. (B) Recombinant Rac1 (25 pmol) was loaded with GTP or GDP (30 μM) and incubated with GST-p120-catenin (5 pmol). Protein complexes were affinity purified with glutathione-Sepharose and analyzed by SDS-polyacrylamide gel electrophoresis and WB with anti-Rac1 and anti-GST mAbs. Rac1 (1 pmol) was included as an internal reference (St). (C) GST-p120-catenin wt isoforms 1 and 3 and the deletion mutants shown in (A) (2 pmol) were incubated with Rac1 (5 pmol). Protein complexes were affinity purified and analyzed with anti-Rac1 and anti-GST mAbs. Internal reference standard (0.5 pmol) for Rac1 was included (St). (D) Autoradiograms from five different experiments performed in triplicate were quantified and the mean ± SD was obtained for each condition. Each of the deletion mutants value is presented relative to that obtained with p120-catenin 1-911 construct. (E and F) GST-p120-catenin wt isoform 1 or 3 (1.5 pmol) were incubated with recombinant Rac1 (E) or cyto-Ecadherin (F) (3 pmol). When indicated, GST-p120-catenin was pre-incubated with a 10-fold excess of cyto-E-cadherin (E) or Rac 1 (F) (15 pmol). Protein complexes were affinity purified and analyzed with anti-E-cadherin, anti-Rac1 and anti-GST. An internal reference standard was included for Rac1 (E) and cyto-Ecadherin (F) (0.5 pmol).

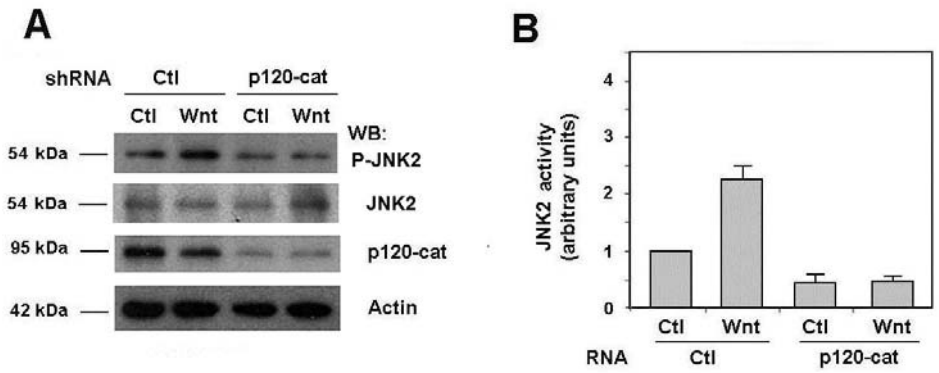
Suppl. Figure 6. Rac1 interacts with ARVCF. GST-p120-catenin wt isoform 1, GST-ARVCF wt and GST-ARVCF (1-382) (2 pmol) were incubated with Rac1 or Vav2 (5 pmol). Protein complexes were affinity purified and analyzed by WB with anti-Rac1, anti Vav2 and anti-GST mAbs. Rac1 (2 pmol) or Vav2 (1 pmol) were included as an internal reference standard (St).

Suppl. Figure 7. Vav2 associates with p120-catenin. (**A**) GST-p120-catenin wt isoforms 1 and 3 and the deletion mutants (2 pmol) were incubated with recombinant Vav2 (5 pmol). Protein complexes were affinity purified with glutathione-Sepharose and analyzed by WB with anti-Vav2 and anti-GST mAbs. Vav2 (1 pmol) was included as an internal reference standard (St). (**B**) Autoradiograms from five different experiments performed in triplicate were quantified and the mean ± SD was obtained for each condition. Each of the deletion mutants value is presented relative to that obtained with p120-catenin 1-911 construct. (**C**) GST-Vav2 or GST (2 pmol) were incubated with recombinant p120-catenin isoform 1 (5 pmol). When indicated the assay was performed in the presence of a 2-fold molecular excess of Rac1 (10 pmol). Protein complexes were affinity purified with glutathione-Sepharose and analyzed by WB with anti-Rac1, anti-Vav2 and anti-GST mAbs. p120-catenin (0.5 pmol) was included as an internal reference standard (St).

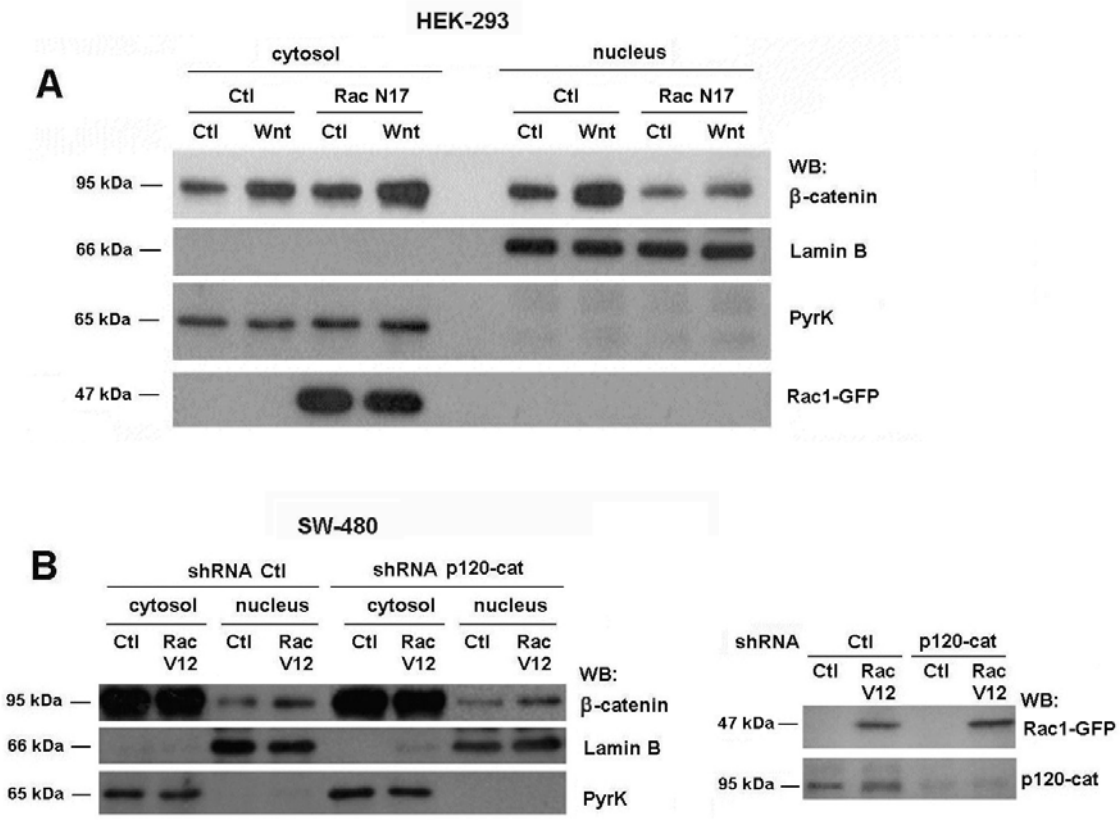
Suppl. Figure 8. p120-catenin translocates from the membrane to the cytosol upon Wnt3a stimulation. SW-480 cells were treated with control or Wnt3a conditioned medium for 2h. Cytosolic and membrane fractions were obtained as described in Material and Methods. The distribution of p120-catenin, Vav2, E-cadherin and Rac1 between both fractions was analyzed by WB. p120-catenin phosphorylation was analysed in both fractions using an antibody specific for phospho-Tyr228. Piruvate kinase was used as a marker for the cytosolic fraction.

Suppl. Figure 9. Xenopus p120-catenin levels after morpholino injection and expression of p120-catenin point mutants in Xenopus embryos. (A) Xp120 protein levels from total embryo extracts isolated at 11-12 stage after morpholino injection were detected via WB using anti-Xp120

polyclonal antibody. Xp120 displayed a SDS-PAGE mobility of ~ 90 kDa. **(B and C)** The expression of mp120 WT, Y112E, Y112F, S268,269D, S268,269A and Y217E mutants was detected via WB using anti-myc antibody from total embryo extracts isolated at 11-12 stage after in vitro transcribed mRNAs injection. Protein loads were assessed by Western blotting samples for GAPDH.



Suppl. Figure 1



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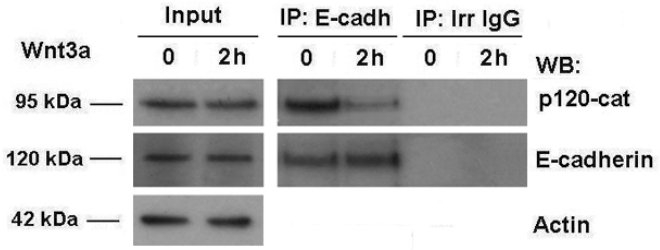

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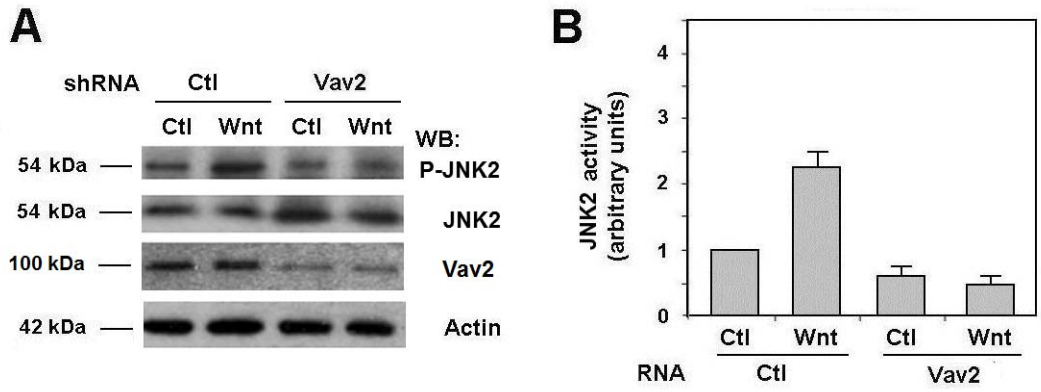

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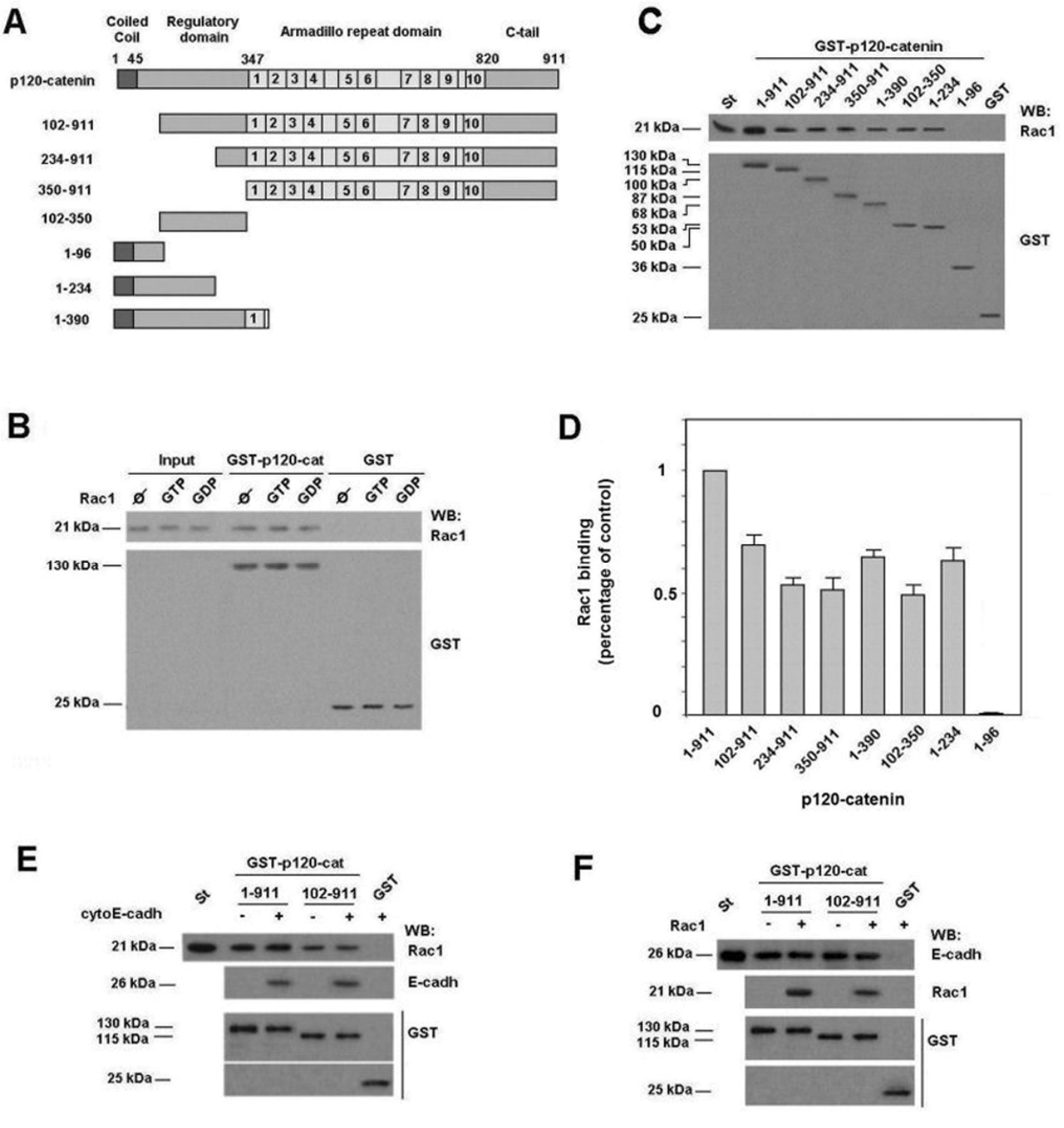

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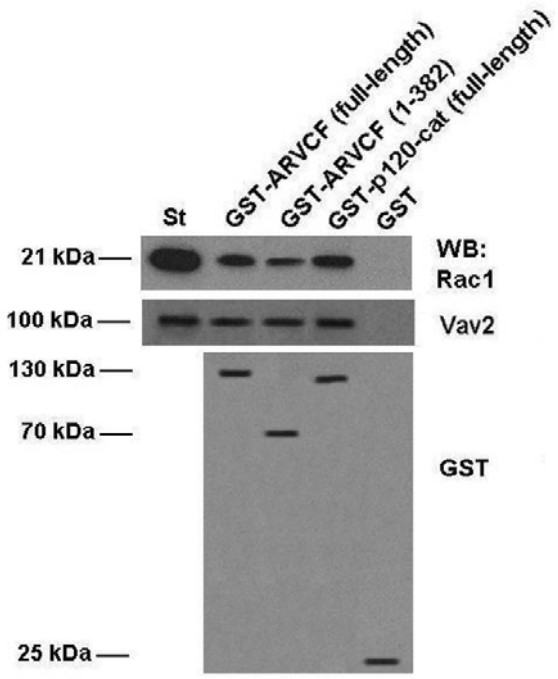

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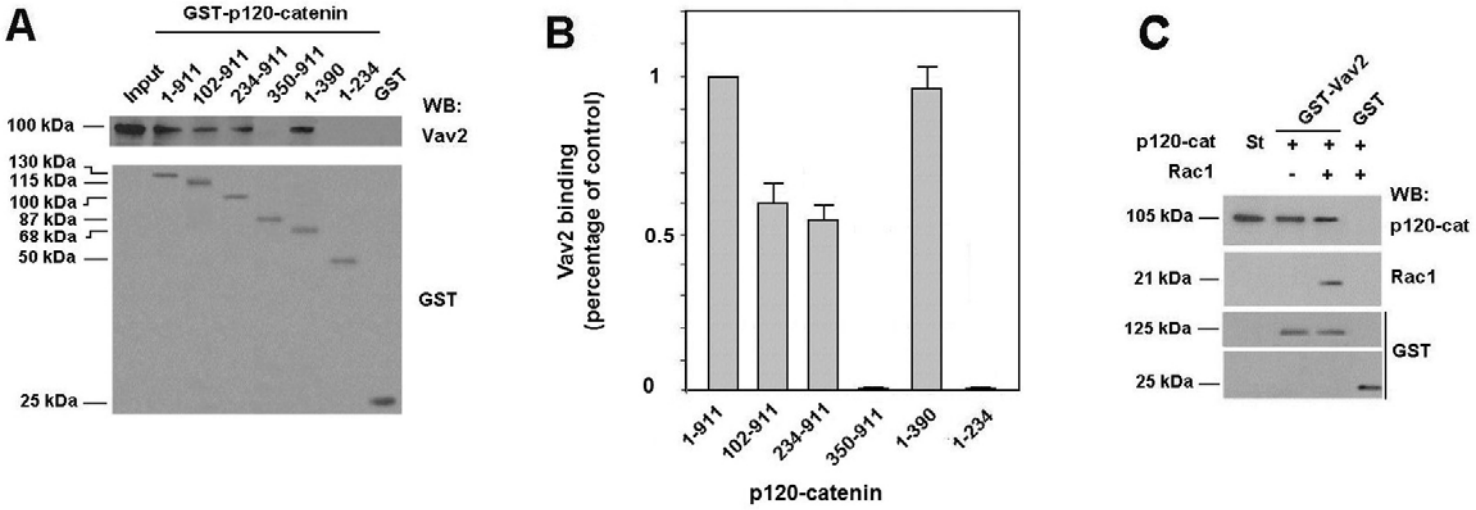

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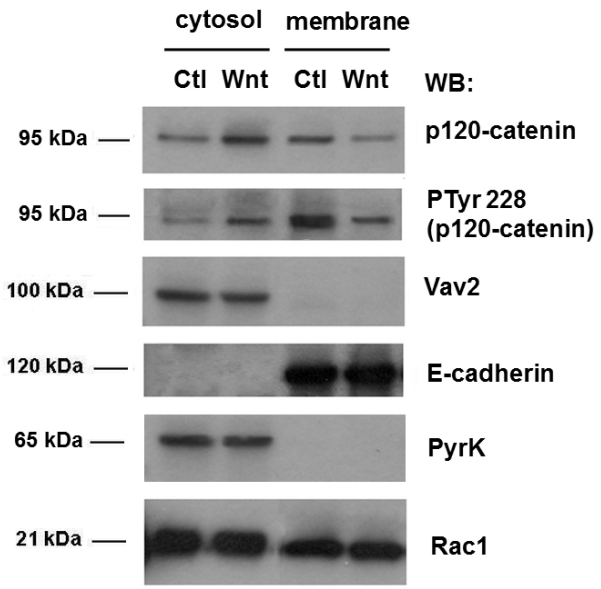

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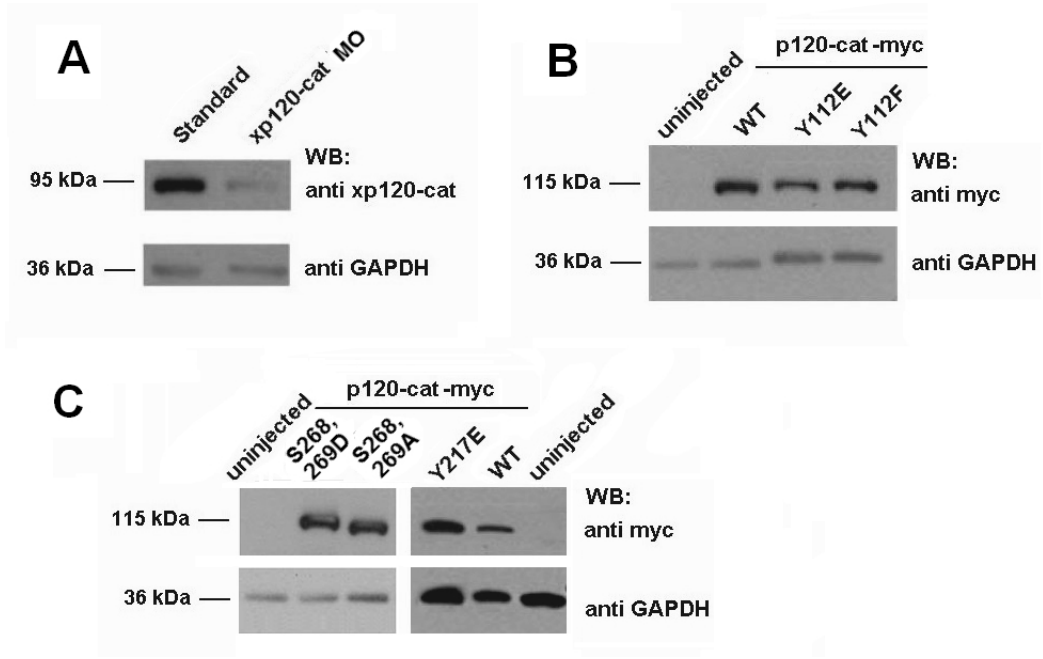

Suppl. Figure 9