SUPPLEMENTAL DATA

Supplementary Fig. 1. Schematic diagram of various forms of Sox9. Nuclear localization signals (NLS) and nuclear export signal (NES) in the Sox9 HMG domain are shown. Sox9ΔC contains amino acids 1-383; Sox9ΔN contains amino acids 104-509; Sox9-NLS* carries the substitutions RRK/GGT at amino acids 120-121 and deletion of PRRRK at amino acids 176-180; Sox9-NLS*NLS (TA) carries a NLS from SV40 large T antigen (TA) at its C-terminus; Sox9^HMG contains a deletion of HMG domain from 103 to 182 amino acids; Sox9^HMG-NLS (TA) contains a deletion of HMG domain, but also carries a NLS from SV40 large T antigen.

Supplementary Fig. 2. *Sox9* was unable to promote degradation of stabilized phosphorylation-resistant β-catenin in SW48 cells (Lanes 1 and 2) or wild type β-catenin in SW480 cells that lack functional APC (Lanes 3 and 4) or in SNU475 cells that lack of Axin (Lanes 5 and 6). SW48, SW480 and SNU475 cells were transiently transfected with Sox9 and 24 hours later, cells were analyzed by Western blot with anti-β-catenin and anti-Sox9 antibodies. The status of the β-catenin phosphorylation was analyzed with phospho-β-catenin-specific (S45 and S33/37) antibodies.

Supplementary Fig. 3. Sox9 promoted nuclear localization of Axin, GSK3β, βTrCP, and CK1α**.** CHO cells were transiently transfected with indicated plasmids or infected with Sox9-adenovirus, and protein sub-cellular localization was analyzed by immunofluorescent cytochemistry. DAPI stained the nucleus. A. Axin (green) was stained with anti-myc, and Sox9 (red) was stained with anti-Flag antibodies. B. CHO cells were infected with *Sox9*-adenovirus and localization of endogenous GSK3β was analyzed with anti-GSK3β antibody (green). Sox9 was detected with anti-Sox9 antibody (red). C. βTrCP (green) was detected with anti-myc antibody and Sox9 (red) was detected with anti-Flag antibody. D. CK1α (green) was stained with anti-HA antibody. In Sox9 (red) expressing cells, more CK1α was found in the nucleus (arrows).

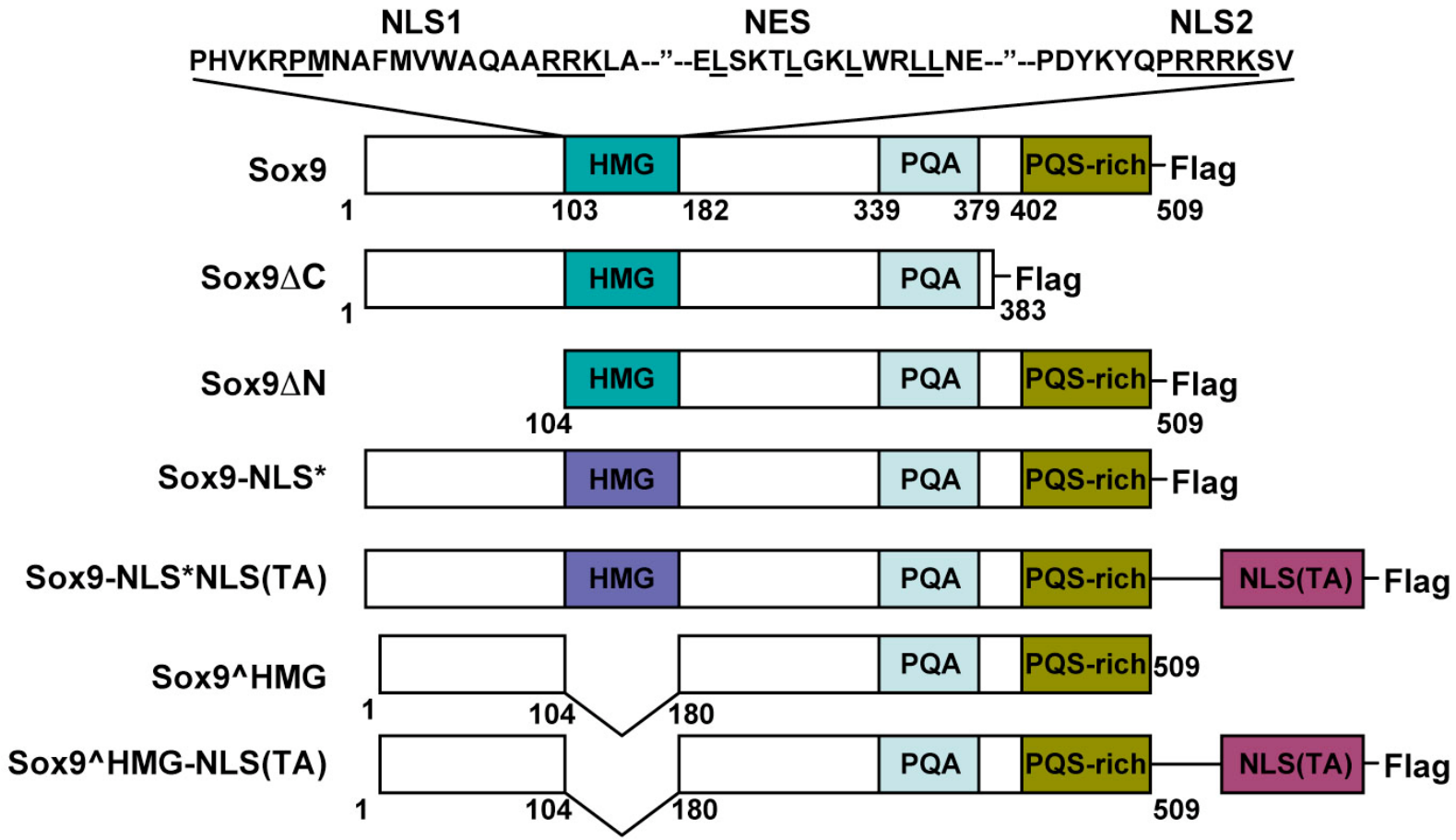
Supplementary Fig. 4. Removal of *Sox9* from primary chondrocytes led to a decreased amount of nuclear Axin, β-catenin, βTrCP and GSK3β**.** Primary chondrocytes were isolated from of *Sox9*$^{c/c}$ mice and infected with *Cre*-adenovirus. Cells were fractionated and analysed by Western blot for the endogenous Axin, β-catenin, βTrCP and GSK3β. Lamins A+C and Tubulin were used as markers for nuclear and cytoplasmic fractions, respectively.

Supplementary Fig. 5. Sox9 bound to βTrCP and promoted its nuclear localization. A. Sox9 coIPed with βTrCP in CHO cells. Cells were transiently transfected with the indicated plasmids and βTrCP was immunoprecipitated by anti-myc antibody. Sox9 was detected by anti-Flag antibody. B. Deletion of C-terminus of Sox9 abolished its binding to βTrCP. Cos cells were transfected with the indicated plasmids and Sox9 was immunoprecipitated with anti-Flag antibody. βTrCP was detected with anti-myc antibody. C. Deletion of F-box in βTrCP did not abolish its ability to coIP with Sox9. CHO cells were transfected with the indicated plasmids and Sox9 was detected in the immuno-complexes with ΔβTrCP with anti-Flag antibody. D. Sox9 formed a complex with βTrCP independently of β-catenin. Sox9 was coIPed with βTrCP in NCI-H28 cells using anti-myc antibody. E. Sox9 formed a complex with GSK3β independently of β-catenin. Sox9 was coIPed with GSK3β in NCI-H28 using anti-HA antibody.

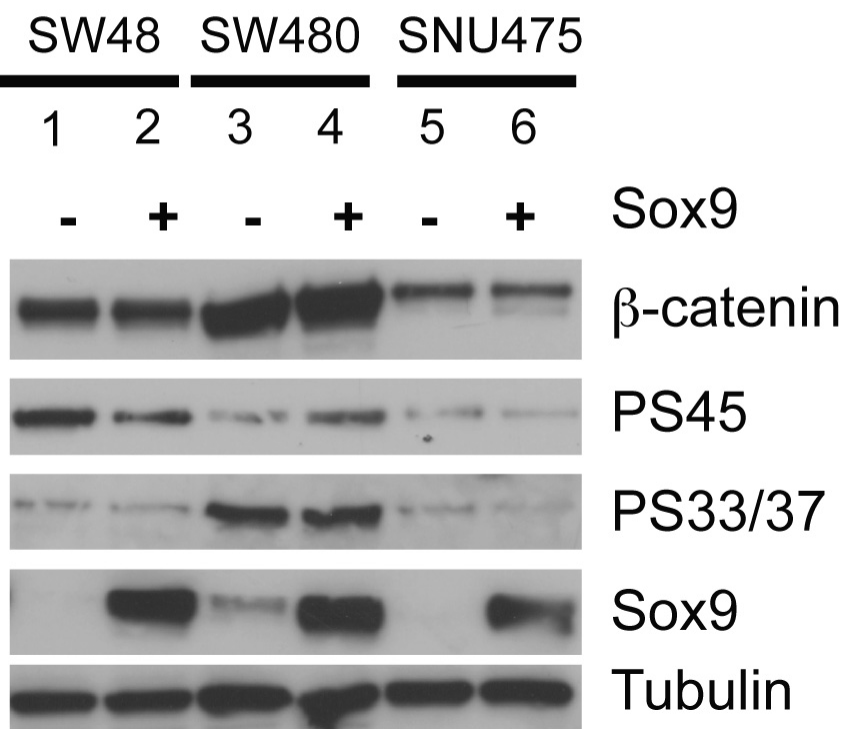
Supplementary Fig. 6. Both cytoplasmic and nuclear forms of Sox9 bound to β-catenin, GSK3β and βTrCP. A. Nuclear Sox9 mutant (Sox9^HMG-NLS(TA)) binds β-catenin more efficiently. Cos cells were transfected with the indicated plasmids. (a) β-catenin was co-IPed with Sox9 and its mutant forms by anti-myc antibody. (b) Amount of IPed β-catenin-myc loaded on gel was normalized by anti-myc antibody. The membrane was stripped and re-probed with anti-Flag

antibody. B. Both cytoplasmic and nuclear forms of Sox9 bind GSK3β. Cos cells were transfected with GSK3-HA and Flag-tagged Sox9 and its mutants. (a) GSK3β was co-IPed with Sox9 and its mutant using anti-HA antibody. (b) Amount of IPed GSK3β-HA loaded on gel was normalized with anti-HA antibody. The membrane was stripped and re-probed with anti-Flag antibody. C. Both cytoplasmic and nuclear forms of Sox9 bind βTrCP. Cos cells were transfected with βTrCP-myc and Flag-tagged Sox9 and its mutants. (a) βTrCP was co-IPed with Sox9 and its mutants using anti-myc antibody. (b) Amount of IPed βTrCP loaded on gel was normalized with anti-myc antibody. The membrane was stripped and re-probed with anti-Flag antibody.

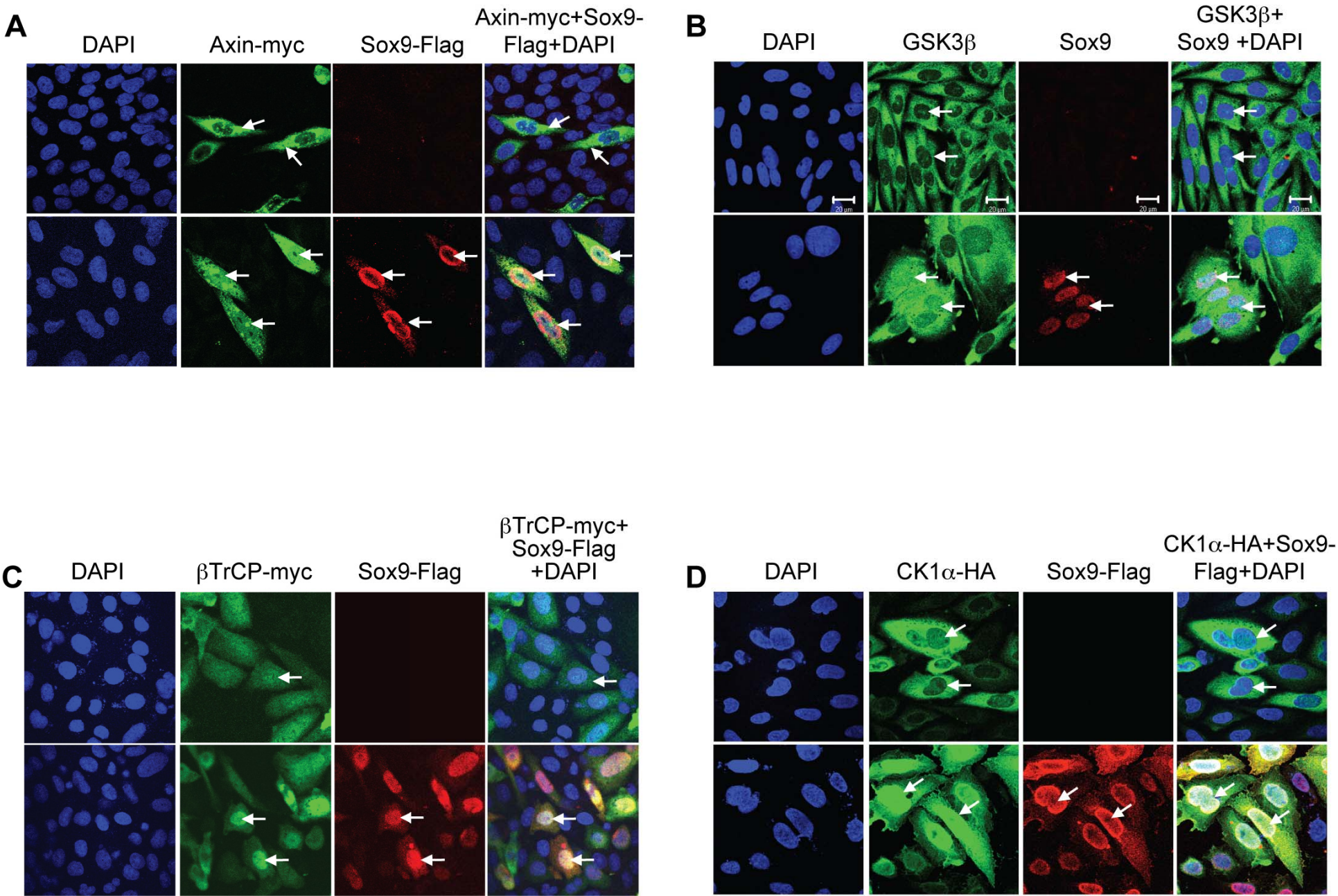
Supplementary Figure 1



Topol_Supplementary Figure 2



Supplementary Figure 3



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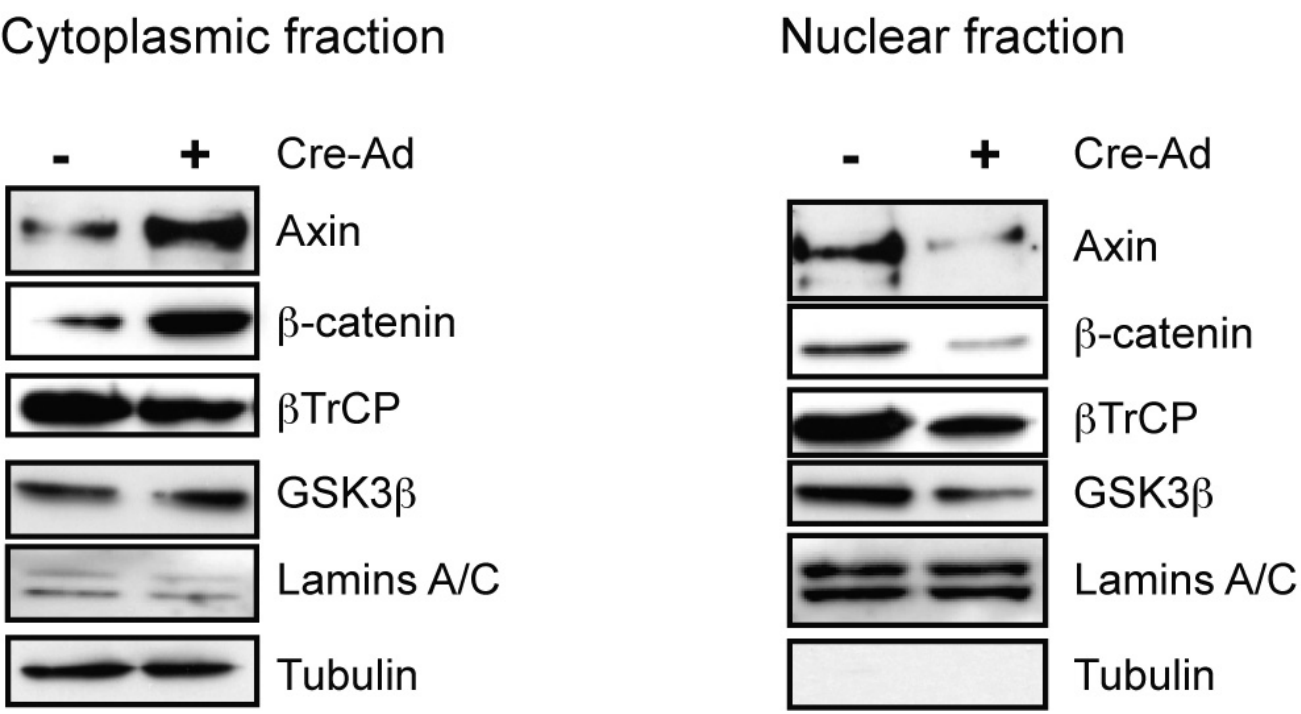

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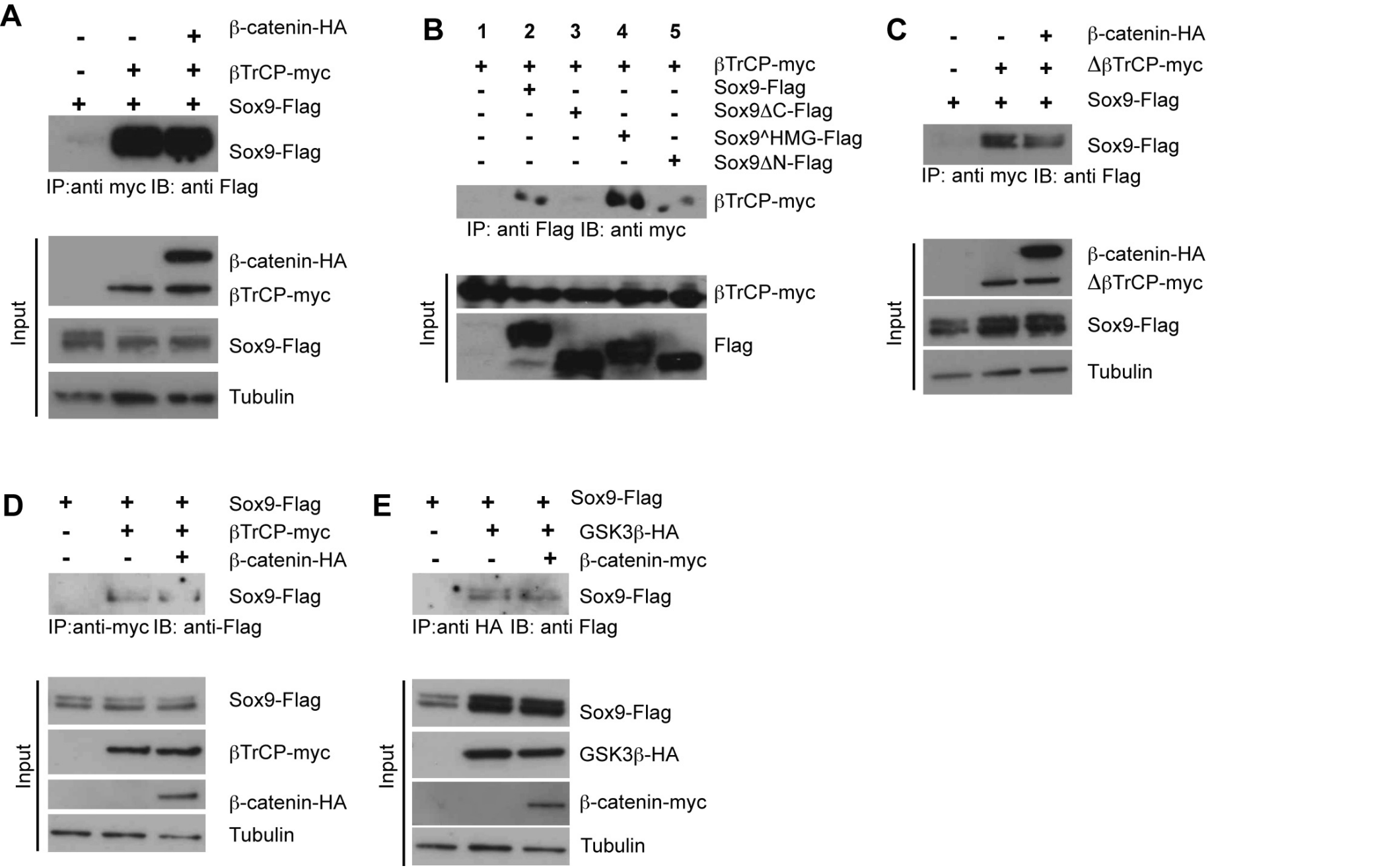

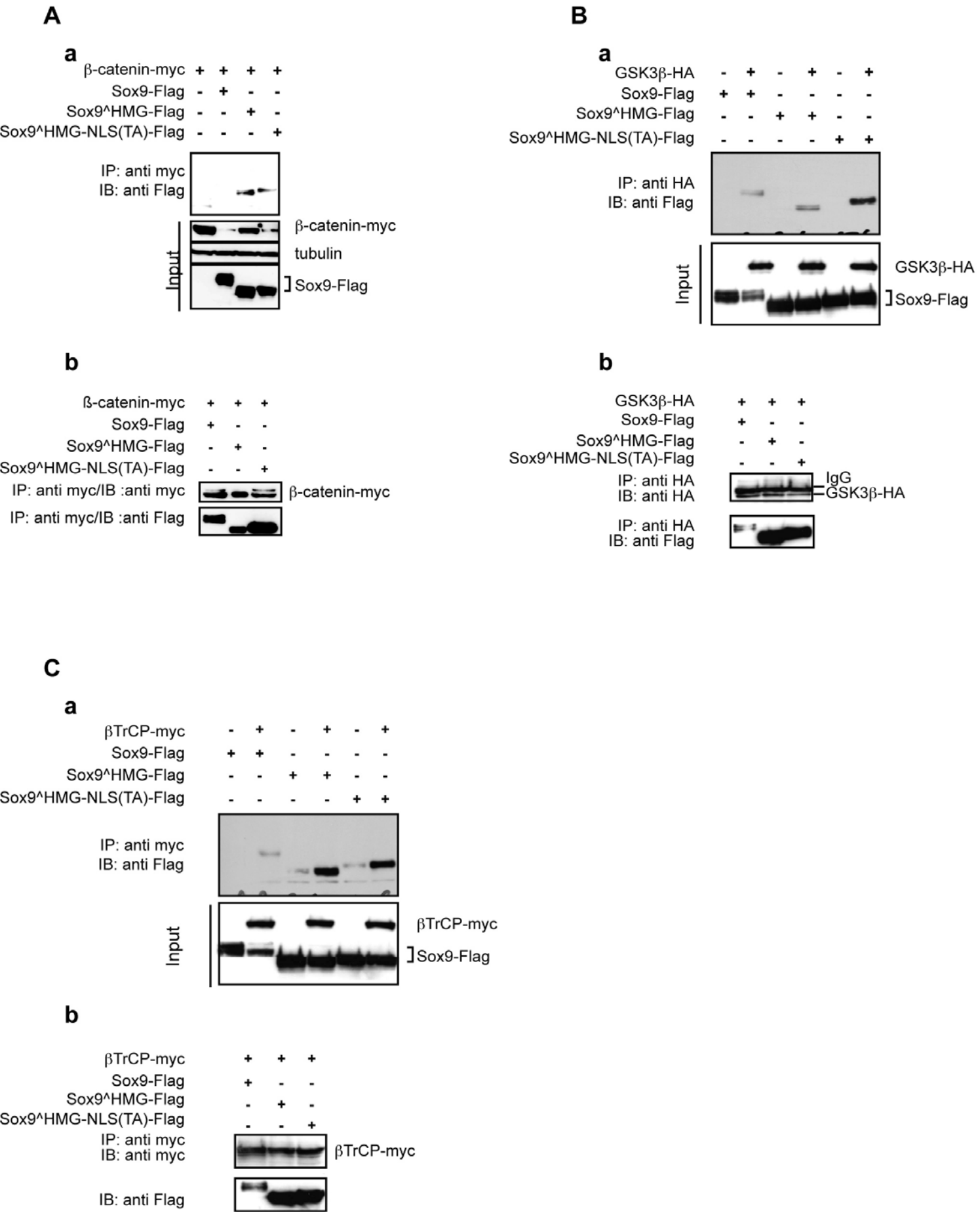
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Sox9^HMG-Flag - - + -
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IB: anti Flag
β-catenin-myc
tubulin
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Sox9^HMG-NLS(TA)-Flag - - +
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β-catenin-myc
IP: anti myc/IB :anti Flag
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IP: anti HA
IB: anti Flag
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IP: anti HA
IB: anti HA
IgG
GSK3β-HA
IP: anti HA
IB: anti Flag
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IP: anti myc
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βTrCP-myc
IB: anti Flag