## **Supplemental Figures**

**Fig. S1:** The CTD of  $\beta$ -catenin shows no obvious interaction partners by metabolic labeling analysis. HEK293T cells were transfected with a construct encoding arm repeats 10-12 and the unstructured CTD (Arm10CTD). After 36 hours, cells were labeled for 3 hours with 200µCi <sup>35</sup>[S]-methionine/cysteine, lysed and immunoprecipitated with the Flag antibody. Untransfected HEK293T cells (parental) served as a control.

**Fig. S2:** β-catenin monoclonal antibody M5.1 recognizes an epitope that is masked in the cytoplasmic compartment of Wnt3a activated L cell fibroblasts. (A) Schematic of β-catenin with antibody recognition domains shown. (B) Epitope mapping of β-catenin CTD antibodies, M5.1 (kindly provided by Jean Luc Teillard, Jussieu University, France) and clone 14 (BD Biosciences) after transfection of HEK293T cells with β-catenin CTD truncation constructs Δ751, 723 and 695. Deletion constructs were made within an oncogenic β-catenin backbone (S33Y) to compensate for the reduced accumulation observed for CTD deleted forms of β-catenin (Frank Kolligs, personal communication; (1)). (C) Immunostaining of cadherin-negative, mouse L cell fibroblasts lacking (control) or stably expressing Wnt3a. Both M.5 and 1.1.1 antibodies recognize a single species that perfectly comigrates with β-catenin in Wnt3a actived L cells (not shown), as previously demonstrated (2).

**Fig. S3:** β-catenin lacking the CTD shows enhanced interaction with phosphorylated ligands.  $10^{6}$  HEK293T cells were transfected with 1.0 (1) and 0.5µg (2) of flag-tagged full length (full) or CTD-truncated β-catenin (Δ695). Empty pcDNA plasmid was transfected as a control. After 36 hours, cells were labeled for 4 hours with 250µCi <sup>32</sup>[P]-orthophosphate, lysed and immunoprecipitated with the flag antibody. Half of each sample was subjected to SDS-PAGE and immunoblot analysis; the other gel was dried and exposed to film.

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## Supplemental References

- Kolligs, F. T., Hu, G., Dang, C. V., and Fearon, E. R. (1999) *Molecular and cellular biology* **19**(8), 5696-5706 Gottardi, C. J., and Gumbiner, B. M. (2004) *J Cell Biol* **167**(2), 339-349 1.
- 2.

Supplemental Figure 1.



## Supplemental Figure 2.

mAb M5.1 mAb 1.1.1 123456789101112C mAb Clone 14 control vector 1, 11, 10, 1723, 1695 В. WB: flag M5.1 Clone 14

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Supplemental Figure 3.

