Supplementary Information to

Src and Fyn define a new signaling cascade activated by canonical and non-canonical Wnt ligands and required for gene transcription and cell invasion

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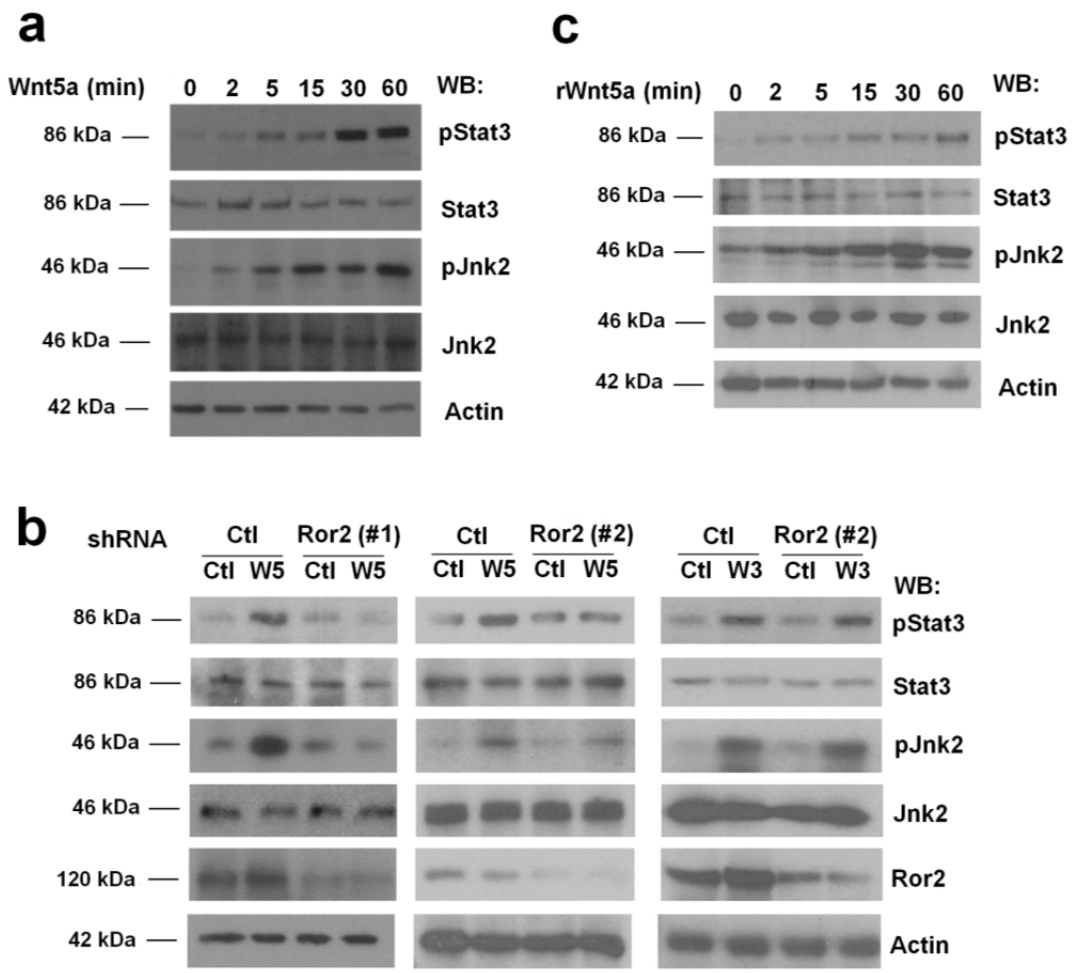
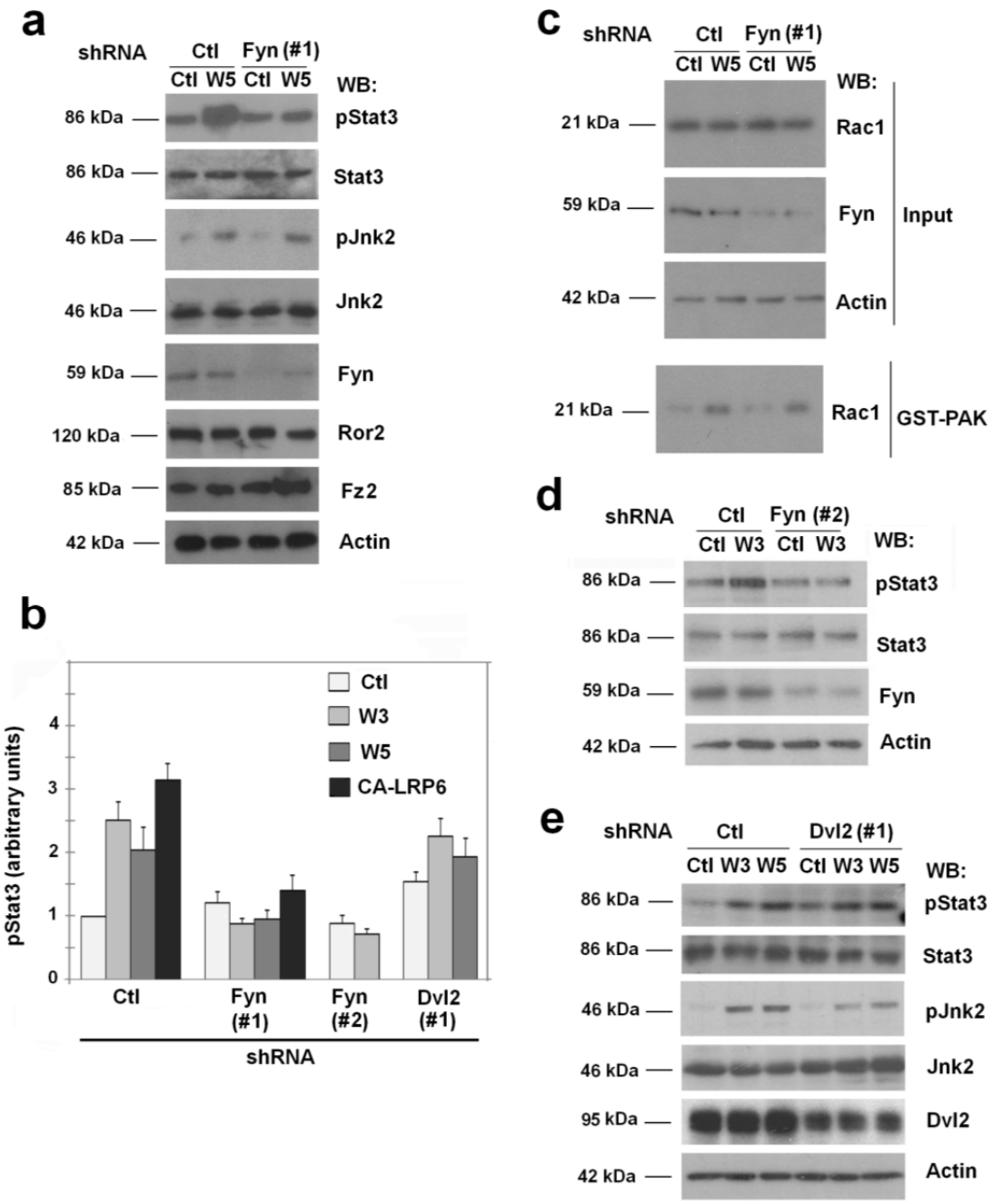
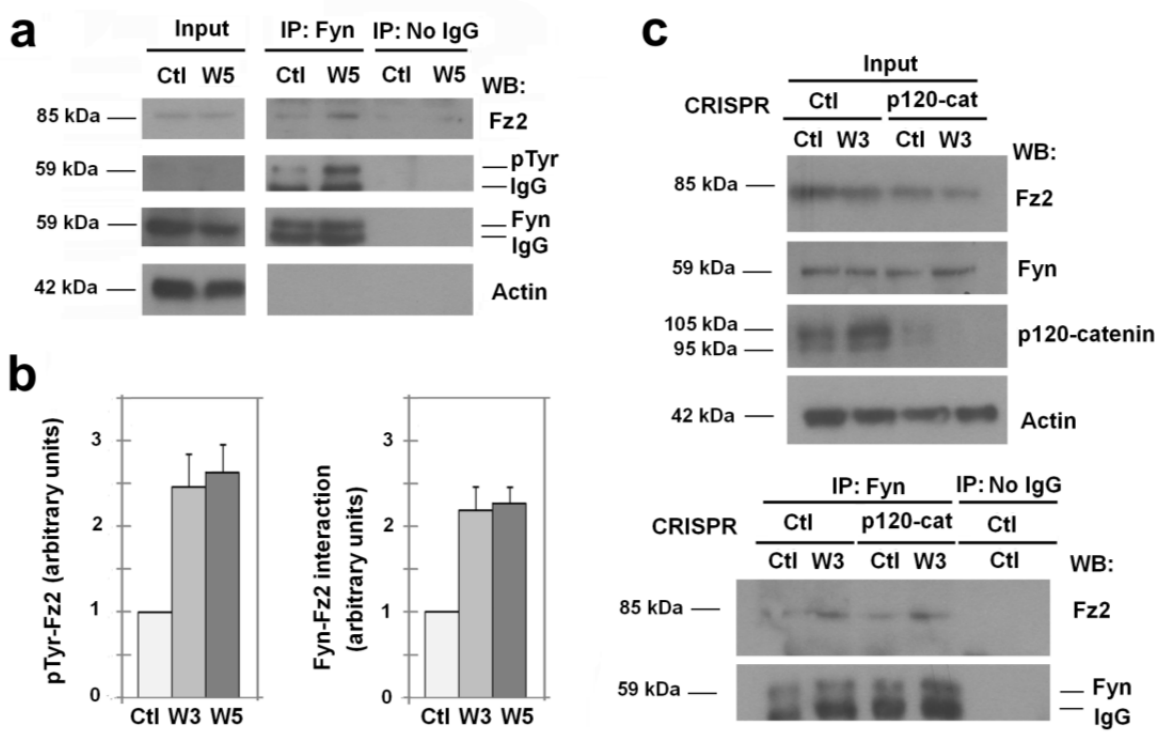


Suppl. Fig. 1. Wnt3a and Wnt5a stimulate Stat3 phosphorylation. (**a**) HEK293T cells were stimulated with control or Wnt5a-conditioned medium for the indicated times. Cells were lysed and proteins were analyzed by WB with specific antibodies. Jnk2 and Stat3 phosphorylation was determined with anti-phospho antibodies against Jnk (Thr183/Tyr185, Thr221/Tyr223) or Stat3 (Tyr705). (**b**) HEK293T cells, depleted of Ror2 with two specific shRNA or transfected with the corresponding control shRNA, were stimulated for 30 minutes with control, Wnt3a- or Wnt5a-conditioned medium as indicated. Cells were lysed and Jnk2 and Stat3 phosphorylation was determined as above. (**c**) Cells were incubated for the indicated times with recombinant Wnt5a (50 ng/ml) and cell extracts analyzed by WB.

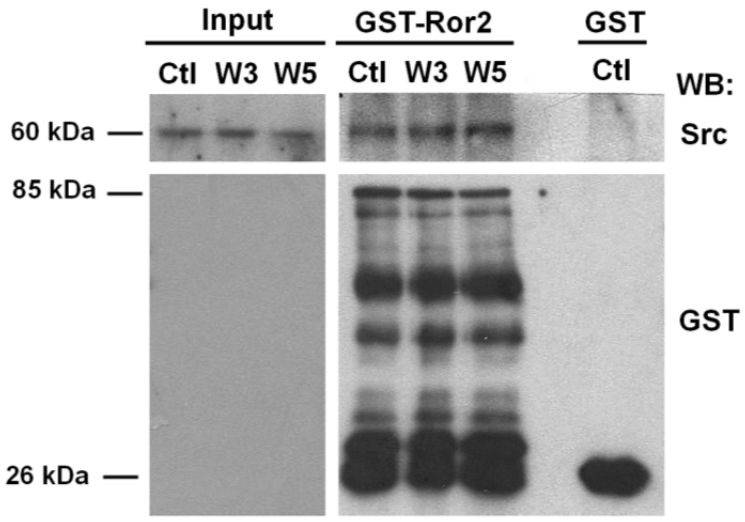


Suppl. Fig. 2. Fyn is required for Wnt3a and Wnt5a-induced Stat3 phosphorylation. (**a**-**d**) HEK293T cells depleted of Fyn using two specific shRNAs or a non-targeting control were treated for 30 min with control, Wnt3a- or Wnt5a-conditioned medium, as indicated. Cells were lysed and Stat3 and Jnk2 phosphorylation was analyzed by WB with specific antibodies; a GST–PAK pull-down assay was performed in **c** and active Rac1 was determined by WB. In **b** a quantification of three independent experiments carried out as in **a**, **d**, **e** and Figures 2a

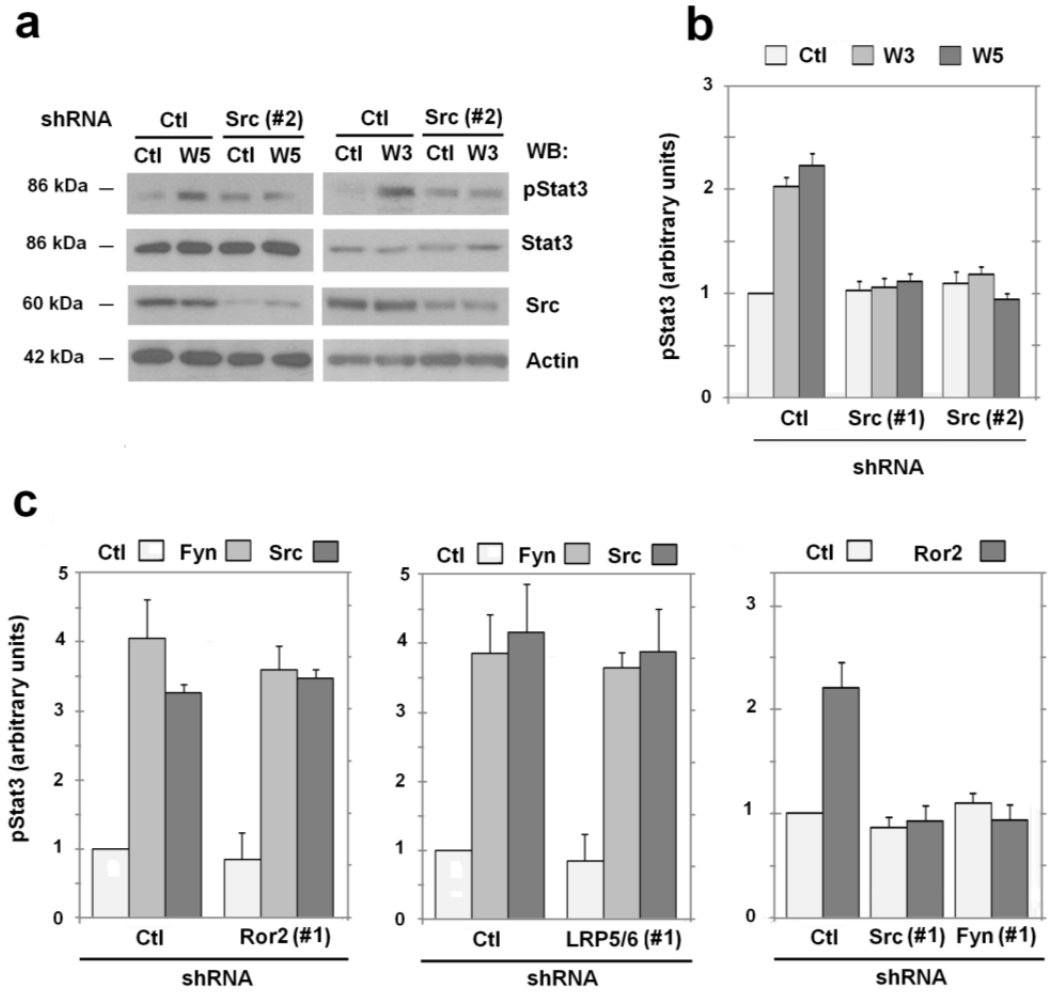
and b is represented. Mean ± SD is shown. (**e**) HEK293T cells were down-regulated of Dvl2 using shRNA #1 or a control shRNA and stimulated with control, Wnt3a- or Wnt5a- conditioned medium for 30 min. Cells were lysed and Stat3 phosphorylation was analyzed by WB.



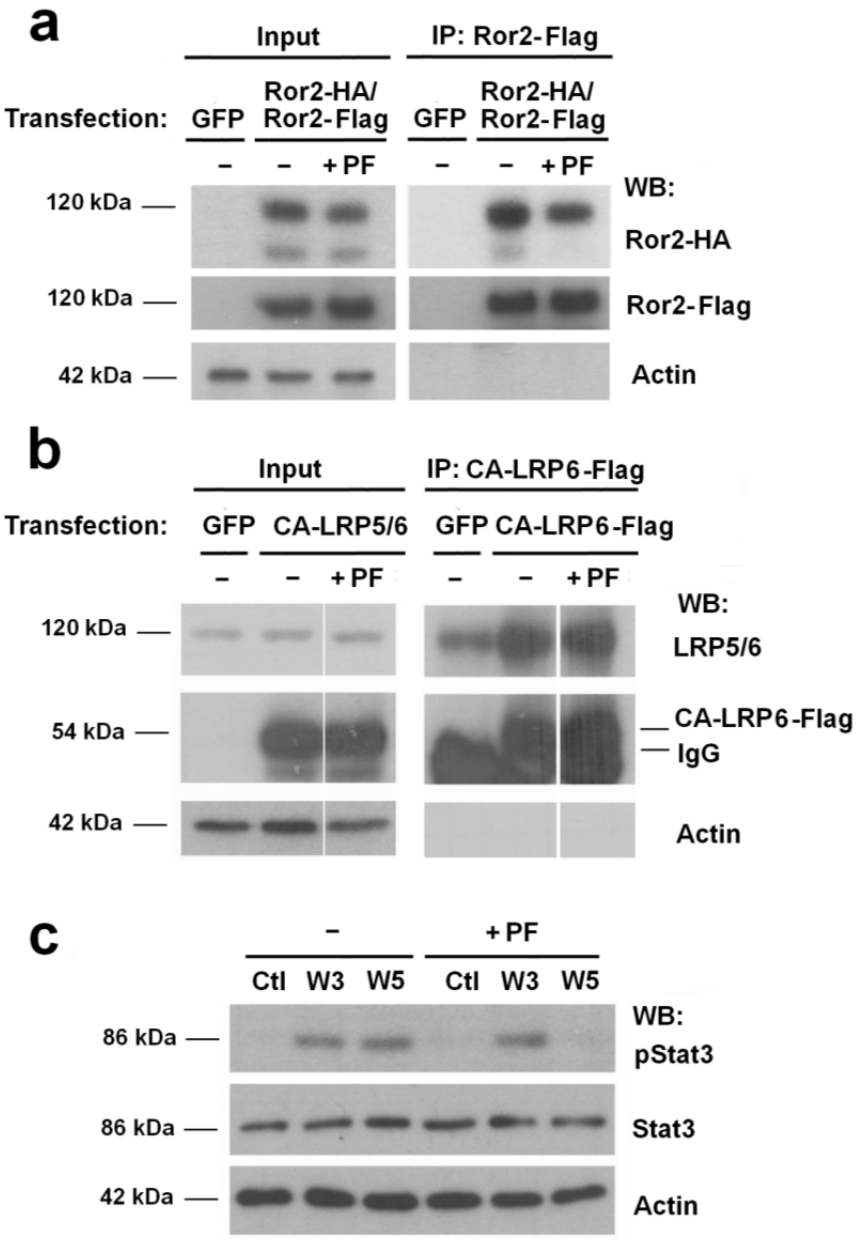
Suppl. Fig. 3. Canonical and non-canonical Wnt promote Fyn phosphorylation and binding to Fz2. HEK293T cells, either control (**a**) or CRISPR-deleted in p120-catenin (**c**) were treated with Wnt3a- or Wnt5a-conditioned-medium for 30 minutes. Fyn was immunoprecipitated from total cell extracts and Fyn phosphorylation and association with Fz2 was determined by WB. In (**b**) signals from the different experiments as in panels **a** and **c** and Figures 2e and f were densitometered and represented. The mean ± SD of three different experiments is shown.



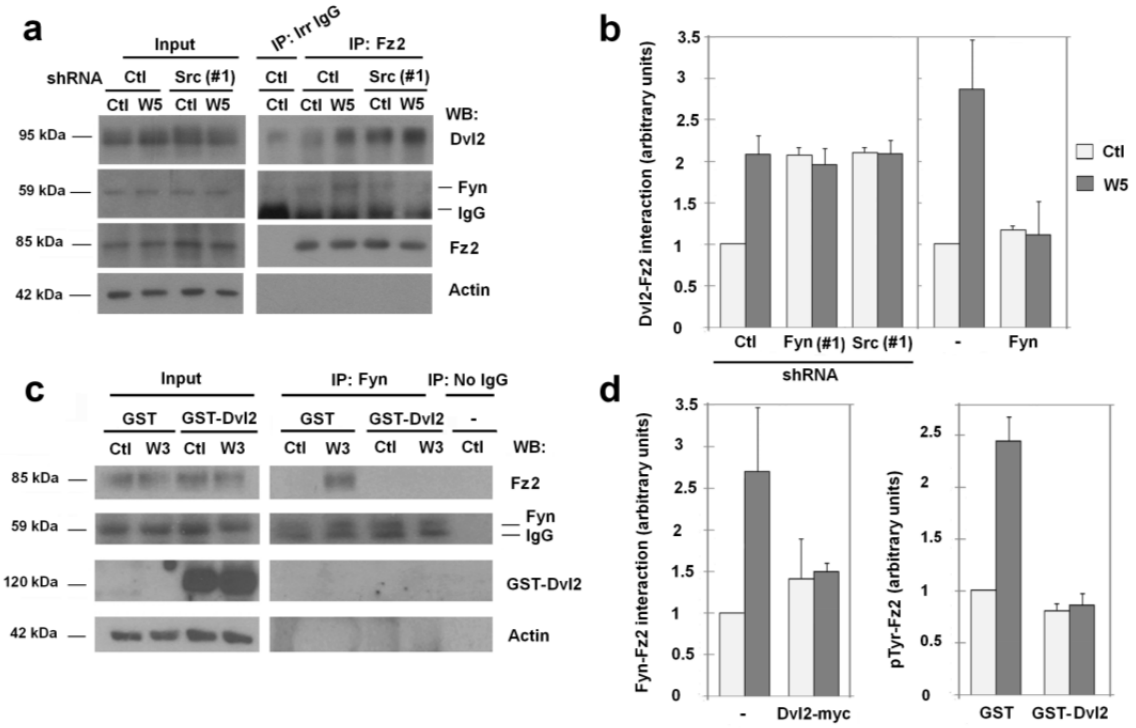
Suppl. Fig. 4. Src directly binds to Ror2 and Wnt does not affect this interaction. A pull-down assay was performed incubating 700 µg of total cell extracts with 10 pmol of GST-cytoRor2 or GST. Protein complexes were affinity purified and analyzed by WB.



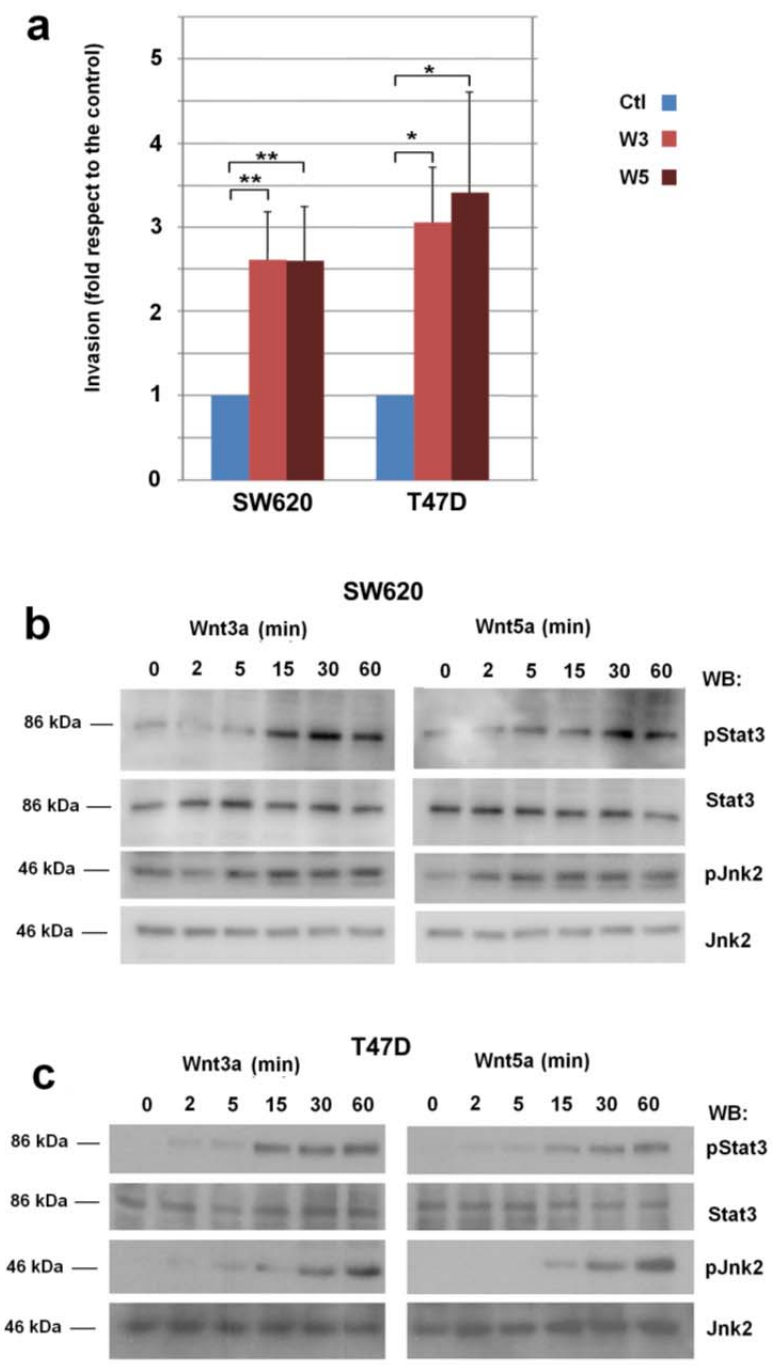
Suppl. Fig. 5. Src is required for Wnt3a or Wnt5a-induced Stat3 phosphorylation. (**a**) HEK293T cells depleted of Src with shRNA #2 or a non-targeting control were treated with control, Wnt3a- or Wnt5a-conditioned medium for 30 min, lysed and Stat3 phosphorylation was analyzed by WB. (**b**) Signal was densitometered and represented. The quantification of three different experiments, as well as those corresponding to Figures 3f and g, is shown (mean ± SD). In (**c**) the quantification of the different experiments performed as in Figures 4a-c is shown (mean ± SD). The ectopically expressed proteins are presented above the graphs.



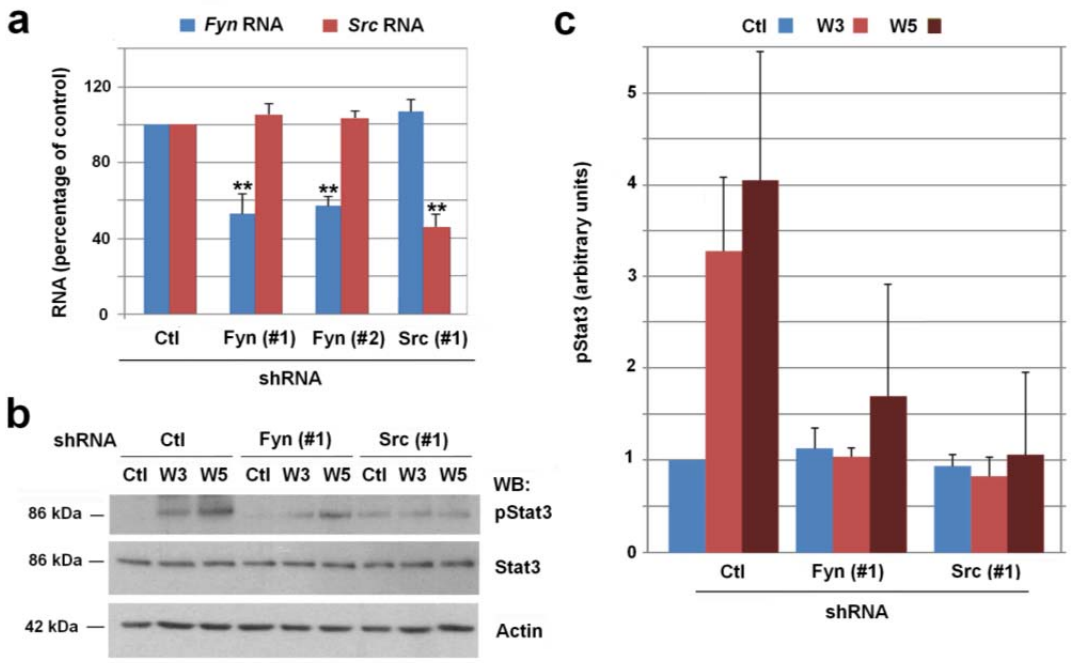
Suppl. Fig. 6. CK1ε activity is required for Ror2 dimerization and Stat3 phosphorylation induced by Wnt5a. (**a**) Ror2-HA, Ror2-Flag or empty vector were overexpressed in HEK293T cells. After 48 hours, cells were treated with PF5006739 (PF) (50 nM) for 1 h. Ror2-flag was immunoprecipitated and association with Ror2-HA was analyzed by WB with anti HA antibody. (**b**) HEK293T cells overexpressing CA-LRP6-Flag or empty vector were treated with PF for 1 h. CA-LRP6-Flag was immunoprecipitated from total extracts with anti-Flag antibody and associated LRP5/6 was determined by WB. (**c**) HEK293T cells were treated with PF for 1 h, total cell extracts were prepared and Stat3 phosphorylation was analyzed by WB.



Suppl. Fig. 7. The two Wnt-induced signaling pathways involving Fz2 interaction with Fyn or Dvl2 are mutually excluding. (**a**) HEK293T cells transfected with a shRNA control or shRNA Src were stimulated with control or Wnt5a-conditioned medium for 30 min. Fz2 was immunoprecipitated from total cell extracts and associated proteins were analyzed by WB. The extent of Src down-regulation is presented in Fig. 3f. (**b**) Quantification of the different experiments of Dvl2-Fz2 association performed as in panel **a** or as in Figures 5a and b. The mean ± SD is shown. (**c**) HEK293T cells overexpressing GST-Dvl2 o a control vector were treated with control or Wnt3a-conditioned medium for 30 min. Fyn was immunoprecipitated from total cell extracts and associated Fz2 was analyzed by WB. (**d**) Quantification of the different experiments of Fyn-Fz2 association and Fz2 tyrosine phosphorylation performed as in panel **c** or as in Figures 5c and d. The mean ± SD is shown.



Suppl. Fig. 8. Wnt3a and Wnt5a also increase cellular invasion and Stat3 phosphorylation in SW620 and T47D tumor cell lines. Colon SW620 (**a, b**) and breast T47D (**a, c**) tumor cell lines were treated with Wnt3a or Wnt5a-conditioned medium for 24 h (**a**) or the indicated times (**b**, **c**); collagen 1 invasion (**a**) or Stat3 phosphorylation (**b**, **c**) were assessed as described. In **a**, **t**he mean ± SD is shown. **, $p<0.01$; *, $p<0.05$.



Suppl. Fig. 9 Fyn and Src are required for Stat3 phosphorylation by Wnt3a or Wnt5a in MSC. (**a**) RNA was isolated from control, Src-, or Fyn-depleted MSC. Expression of *Fyn* (blue bars) and *Src* (red bars) RNA was assessed by quantitative RT-PCR. Results are presented as mean ± SD from three independent experiments. **, $p<0.01$. (**b**) MSC, control, Src-, or Fyn-depleted MSC, were treated with control, Wnt3a- or Wnt5a-conditioned medium for 30 min, lysed and Stat3 phosphorylation was analyzed by WB. (**c**) A quantification of the three independent Stat3 phosphorylation analysis performed in **b** is represented (mean ± SD).

Gene	Primer Sequence (5'-3')
AXIN2 (*Homo sapiens*)	FW: TGTCTTAAAGGTCTTGAGGGTTGAC RV: CAACAGATCATCCCATCCAACA
SIAH2 (*Homo sapiens*)	FW: TTTGCCATCGTCCTGCTCAT RV: GGCTGTGTCGAAAACAAG
CCND1 (*Homo sapiens*)	FW: CTCAGACTTGCGCGTCACAG RV: CAGAACACGGCTCACGCTTA
MMP7 (*Homo sapiens*)	FW: TGTGGGGCAAAGAGATCCCC RV: CCTCGCGCAAAGCCAATC
THBS1 (*Homo sapiens*)	FW: GAGCTGGCCTGCGAGTTC RV: TGTGTGTACCGGAGCGC
MMP9 (*Homo sapiens*)	FW: TTCAGGGAGACGCCCATTTC RV: TGGGTGTAGAGTCTCTCGCT
MMP13 (*Homo sapiens*)	FW: AAGGACCCTGGAGCACTCAT RV: CCTGGACCATAGAGAGACTGGA
SNAI1 (*Homo sapiens*)	FW: GTGCCTCGACCACTATGCC RV: GCTGCTGGAAGGTAAACTCTGG
TCF7 (*Homo sapiens*)	FW: ACCAAGAATCCACCACAGAGAC RV: TCACATCTGCACCAAGGCAA
PUM1 (*Homo sapiens*)	FW: GACCAGCAGAATGAGATGGTTC RV: CATAAGGATGTGTGGATAAGGCA
Fyn (*Mus musculus*)	FW: GGAAAAAGGATCCGGAAGAG RV: GCAGGCTCTCACAGGTTTTC
Src (*Mus musculus*)	FW: GCTGTATGCTGGTGTCGG RV: CAGTGGGGTAGCCGCAAATA
Pum1 (*Mus musculus*)	FW: CAGGTAATTAACGAGATGGTGCG RV: ACGGGTGCGTAGACAAAGC

Table S1: Oligonucleotides used in the RT-PCR analysis.