Supplementary Information

PAPTi: A Peptide Aptamer Interference Toolkit for Perturbation of Protein-Protein Interaction Network

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Supplementary Methods for phage screening of

1. Construction of the FNDY library

The sequences of the wild type FNDY ORF is:

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atggccgtttctgatgttccgcgtaagctggaagttgttgctgcaccccgactagcctg
 M   A   V   S   D   V   P   R   K   L   E   V   V   A   A   T   P   T   S   L

ctgatcagctgggatgctcctgctgttaccgtgcgttattaccgtatcacgtacggtgaa
 L   I   S   W   D   A   P   A   V   T   V   R   Y   Y   R   I   T   Y   G   E
                             BC Loop
accggtggtaactccccggttcaggagttcactgtacctggttccaagtctactgctacc
 T   G   G   N   S   P   V   Q   E   F   T   V   P   G   S   K   S   T   A   T

atcagcggcctgaaaccgggtgttgactataccatcactgtatacgctgttactggccgt
 I   S   G   L   K   P   G   V   D   Y   T   I   T   V   Y   A   V   T   G   R
                                                                        FG
ggtgacagcccagcaagcaagccaatctcgattaactaccgtacc
 G   D   S   P   A   S   K   P   I   S   I   N   Y   R   T
   Loop
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To construct the library, degenerated oligonucleotides with randomized codons were incorporated to replace the BC and FG loop (underlined) in the wild type FN3 scaffold. 4 oligonucleotides (sequences provided below) were synthesized and assembled to create the randomized BC/FG loop FNDY ORF.

P1:
5'GCCATGGCCGTTTCTGATGTTCCGCGTAAGCTGGAAGTTGTTGCTGCGACCCCG
ACTAGCCTGCTGATCAGCTGGGATGCTCCT3'

P2:
5'CCACCGGTTTCACCGTACGTGATACGGTAATAMNNMNNMNNMNNMNNAGGAGCA
TCCCAGCTGATCAG3'

P3:
5'GAAACCGGTGGTAACTCCCCGGTTCAGGAGTTCACTGTACCTGGTTCCAAGTCT
ACTGCTACCATCAGCGGCCTGAAACCGGGTGTTGACTATACC3'

P4:
5'GGCGGCCGCTGGTACGGTAGTTAATCGAGATTGGCTTGCTMNNMNNMNNMNNMN
NAGTAACAGCGTATACAGTGATGGTATAGTCAACACCCGGG3'

To assemble the library, 20μg of P1 and P2 oligos were annealed in 20mM Tris, pH7.6. The double strands DNA (dsDNA) of the annealed P1/P2 were generated by adding Klenow DNA polymerase (New England Biolabs) following the

manufacturer's instruction. At the same time, P3 and P4 oligos were annealed and treated with Klenow DNA polymerase in the same way as P1/P2. The resulting two dsDNA fragments obtained from P1/P2 and P3/P4 were then digested with AgeI and ligated by T4 DNA ligase (New England Biolabs) followed by agarose gel extraction (Qiagen gel extraction kit) to obtain the ligated library FNDY ORF. The FNDY library ORF fragments were then digested by NcoI/NotI and inserted into pHEN phagemid by overnight T4 DNA ligation (New England Biolabs). To generate a phage display library, ligated pHEN-FNDY constructs were then introduced into XL1 Blue electro competent cells by electroporation (Gene Pulser, Bio-Rad) following the manufacturer's instruction.

2. Selection of Peptide Aptamers

For phage culture, XL1 Blue library cells after electroporation were cultured in 100 mL LB for 20 hours and centrifuged at 10,000G, 4^0C for 10 minutes to collect supernatants containing the phage particles. To set up the peptide aptamer selection experiment, freshly obtained phage supernatant and proteins were used. Target proteins were biotinylated (using biotinylation kit from Sigma-Aldrich) and immobilized (2-4μg per well) on streptavidin coated 96-well ELISA plates (Nunc), followed by incubation with TBS+1% BSA blocking buffer. For phage binding, 100μl phage library were added into a target protein coated well (2 wells were used for each protein in each selection) and incubated at 4^0C for 8 hours. After phage binding, the wells were washed with TBST 10~15 times to remove the non-binding phage particles. The remaining target bound phages were rescued by adding 100μl of log phase XL1 blue/M13 helper E. Coli cells. The phage-absorbing E. Coli cells were collected and cultured in LB for 20 hours to produce phage particles. The resulting phage pool was then used for the second round of selection following the same method as described for the initial selection. After 3 rounds of selection and enrichment, phage-absorbing XL1 Blue cells were plated on LB/Agar plates to isolate individual colonies. The correspondent phagemid DNA was obtained by plasmid mini-prep and sequenced.

3. ELISA validation of individual FNDY phage clones

To validate individual FNDY clones, 5mL LB cultures of single colonies picked from the 3rd round peptide aptamer selection were prepared. After 20 hours of shaking at 37^0C, 225 rpm, individual cultures were centrifuged at 10,000G to collect the phage supernatant. To perform the ELISA binding assay, target proteins were immobilized (2~4 4μg/well) in a 96-well ELISA plate. 100μl phage supernatant from each individual colony was added into the well and incubated at 4^0C for overnight. After binding, the wells were washed with TBST, followed by incubating with anti-M13-HRP antibody (Abcam) at room temperature for 1 hour. After washing with TBST, the binding activity was measured by adding the chromogenic substrate TMB (Sigma) and the HRP activity was quantitated at O.D 450 nM with a plate reader (Molecular Devices). The validated positive FNDY clones were further collected and archived for following cell-based characterizations.

Table S1. Sequences of FNDY clones described in this study

Wild type FN3 scaffold ORF (The BC and FG loops are marked underlined):
MAVSDVPRKLEVVAATPTSLLISWDAPAVTVRYYRITYGETGGNSPVQEFTVPGS KSTATISGLKPGVDYTITVYAVTGRGDSPASKPISNYRT

Sequences of the BC and FG loops of selected FNDY clones:

	BC	FG
Wt:	AVTVR	GRGDSPA
B2:	IERSD	QATAK--
B4:	SDKTR	GLWAR--
B5:	DVYSI	RWSYG--
B8:	DVKWN	QRNLN--
DX1:	PATYK	PWSAM--
DX5:	GSFLK	AVVIK--
DX7:	MNPKR	WRTNR--
DP4:	QAVYS	GPSWA--
NAK1:	EVPML	GPNSM--
NAK2:	ELVHF	DYYGL--

Figure S1. FNDY-B8 inhibits Wnt signaling in colorectal cancer cell DLD1.
FNDY-B8 inhibits TOPFLASH reporter activity as effectively as the dnTCF4 construct in the human colerectal cancer cell line DLD1.

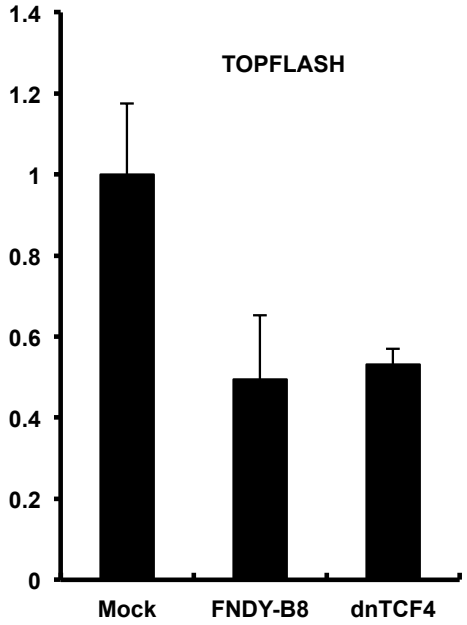


Figure S2. TOPFLASH reporter assay of Dsh and β-cat FNDY peptide aptamers. (A) Increasing amount of Dsh (from 5ng to 125 ng of pCMV-Dsh palsmid/well) can override the inhibitory activity of FNDY-DP4, as assayed using the TOPFLASH Wnt pathway reporter in HEK293T cells cultured in a 96-well plate. **(B)** Similarly, increasing doses of β-cat can override the inhibitory activity of FNDY-B5 and FNDY-B8.

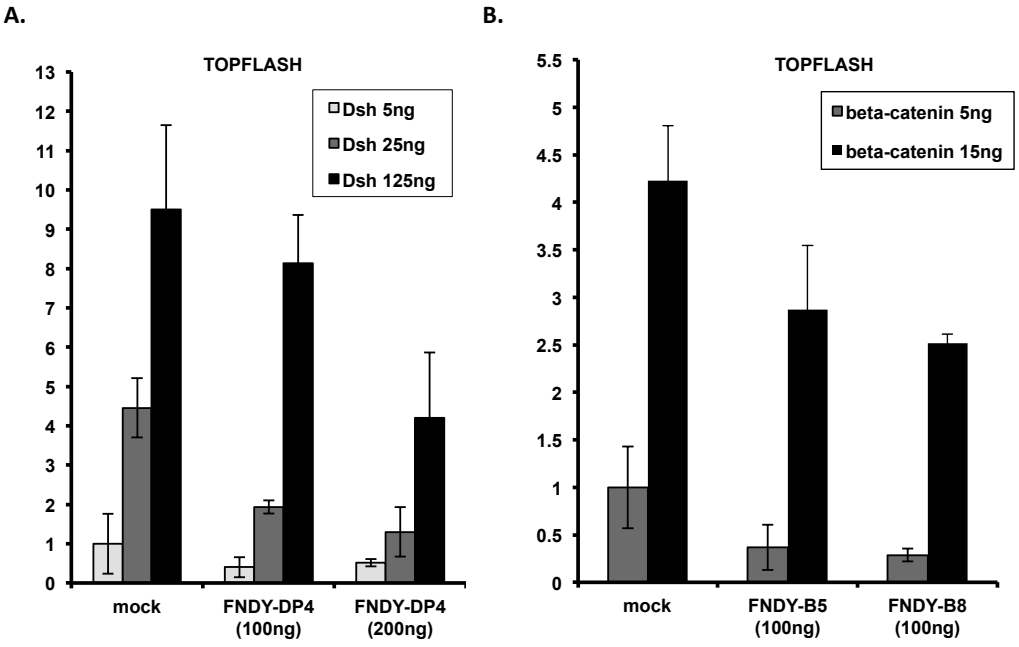
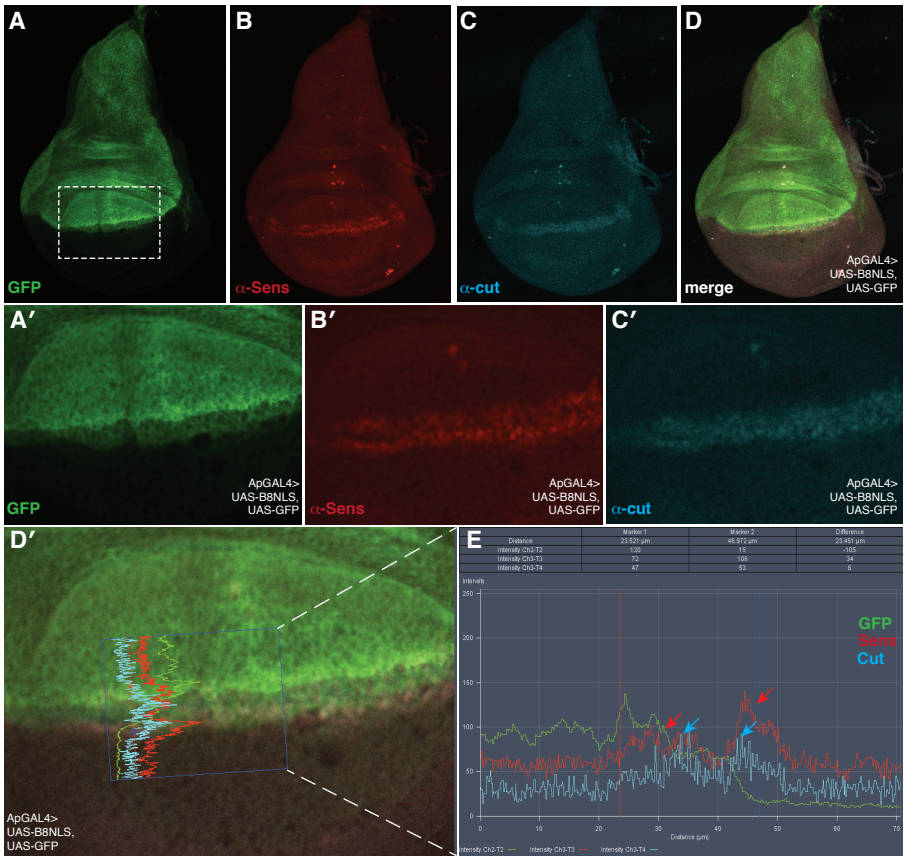
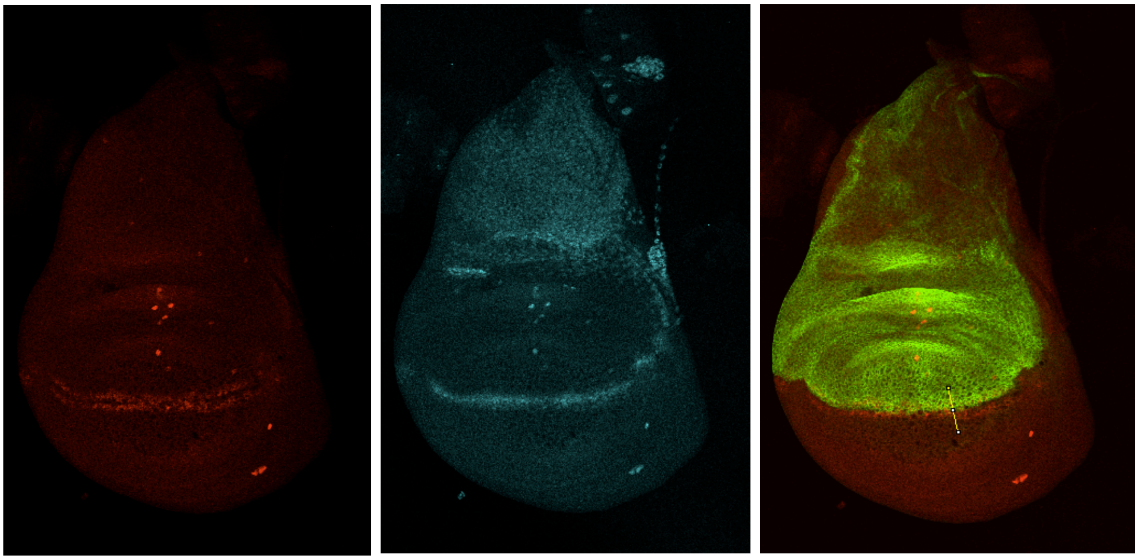


Figure S3. Staining of Cut and Senseless in the wing discs

(A) The expression of B8-NLS does not influence cut staining on either side of D/V boundary even though there is reduced senseless in the dorsal compartment, thereby suggesting a specific effect of B8-FNDY in influencing expression of Wg targets rather than Notch targets. (A'-D' are zoomed-in images of A-D and panel E is the quantification of fluorescence intensities that reveal suppression of Senseless (sens, in red) in "high" green areas (where B8 is being expressed), whereas there is no differential expression of cut (cyan).



(B) Similarly, staining from a different wing disc showed that B8-NLS does not influence cut staining on either side of D/V boundary but reduced senseless level in the dorsal compartment where the Ap-Gal4 is expressed. These results suggest a specific effect of B8-FNDY in influencing expression of Wg targets rather than Notch targets.



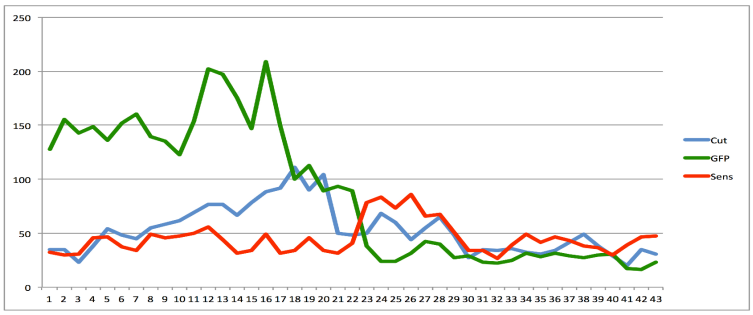


Figure S4. GST pull down of mammalian expressed FNDY aptamers

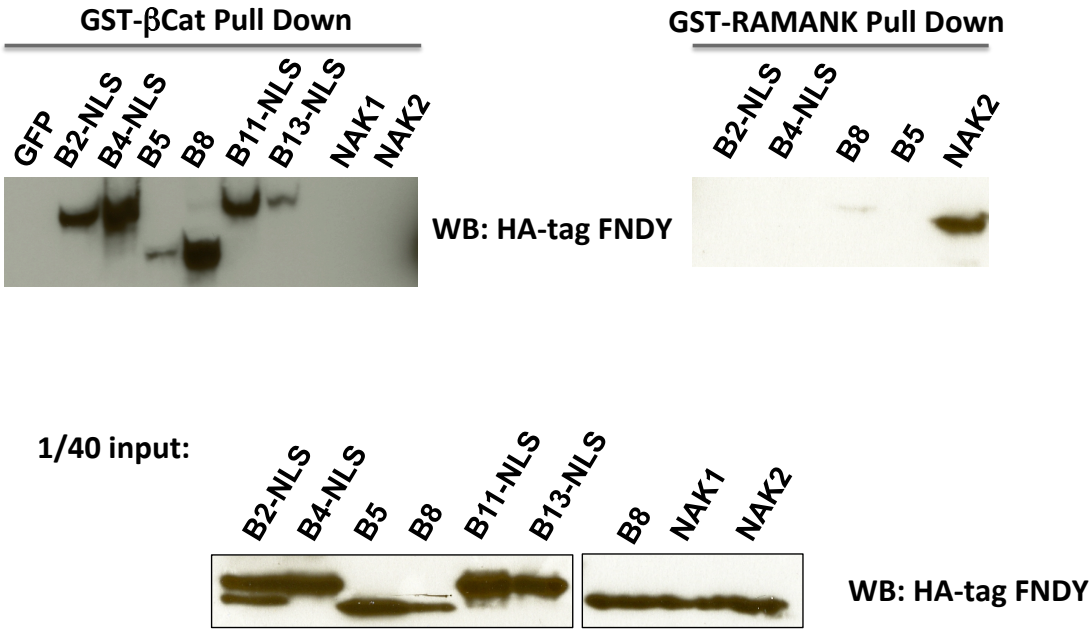


Figure S5. TOPFLASH and CSL-Luc reporter assay used in this study. (A) Structure of Notch ANK/CSL/MAML in complex with DNA[9]. (Cyan: Notch ANK Repeats, Green: CSL, Megenta: MAML, Orange: DNA. Picture based on PDB: 2F8X). The ANK repeats region of Notch 1 was used in this study. (**B)** HEK293T cells is responsive to Notch1 overexpression and is sensitive to γ-secretase inhibitor. The Notch pathway luciferase reporter CSL-Luc (containes 4XCSL binding site in the promoter region) was tested in HEK293T cells. (Gray bar: Basal CSL-Luc activity in HEK293T cells, Black bars: HEK293T cells transfected with ΔEC-Notch1 and treated with either DMSO or 5μM DAPT). HEK293T cells are Notch responsive and therefore suitable for testing the crosstalk between Notch and Wnt pathways.

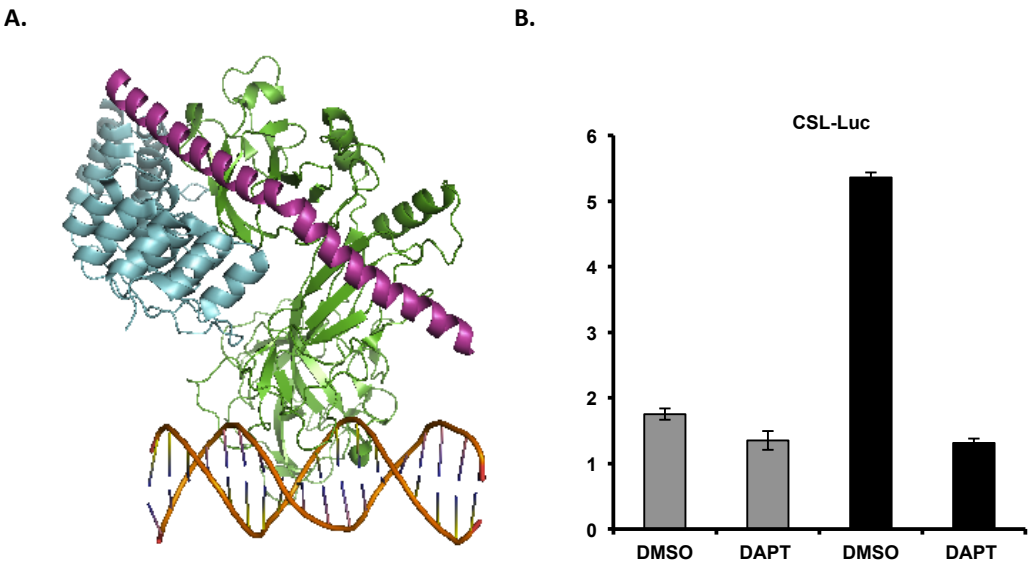
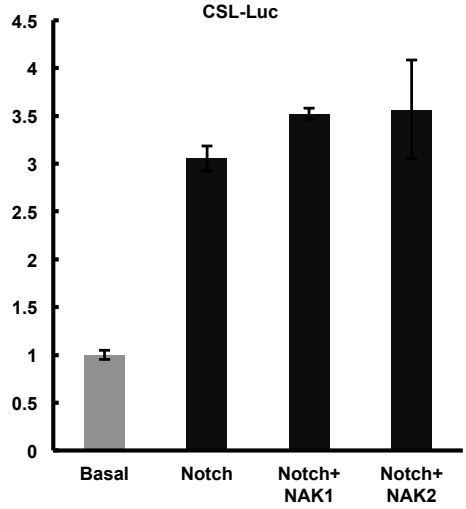


Figure S6. CSL-Luc reporter assay of FNDY-NAK1 and FNDY-NAK2. FNDY-NAK1 and FNDY-NAK2 do not interfer with the canonical Notch transcriptional activity. Possibly FNDY-NAK1 and FNDY-NAK2 target the interface that is not responsible for the assembly of the Notch/CSL/MAML transcriptional complex. Alternatively, Bianco *et al.*[10] have demonstrated that the interaction between Notch RAM and CSL BTD domains largely contributes to the binding energy for the assembly of the Notch/CSL/MAML transcriptional complex. This finding could explain why targeting the ANK region alone by FNDY-NAK1 and FNDY-NAK2 did not perturb canonical Notch signaling.



References

1. Karatan, E. et al. Molecular recognition properties of FN3 monobodies that bind the Src SH3 domain. *Chem Biol* **11**, 835-44 (2004).
2. Koide, A., Bailey, C.W., Huang, X. & Koide, S. The fibronectin type III domain as a scaffold for novel binding proteins. *J Mol Biol* **284**, 1141-51 (1998).
3. Smith, G.P. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science* **228**, 1315-7 (1985).
4. Clevers, H. & Nusse, R. Wnt/beta-Catenin Signaling and Disease. *Cell* **149**, 1192-205 (2012).
5. Sampietro, J. et al. Crystal structure of a beta-catenin/BCL9/Tcf4 complex. *Mol Cell* **24**, 293-300 (2006).
6. Zheng, N. et al. Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex. *Nature* **416**, 703-9 (2002).
7. Wu, G. et al. Structure of a beta-TrCP1-Skp1-beta-catenin complex: destruction motif binding and lysine specificity of the SCF(beta-TrCP1) ubiquitin ligase. *Mol Cell* **11**, 1445-56 (2003).
8. Caussinus, E., Kanca, O. & Affolter, M. Fluorescent fusion protein knockout mediated by anti-GFP nanobody. *Nat Struct Mol Biol* **19**, 117-21 (2011).
9. Nam, Y., Sliz, P., Song, L., Aster, J.C. & Blacklow, S.C. Structural basis for cooperativity in recruitment of MAML coactivators to Notch transcription complexes. *Cell* **124**, 973-83 (2006).
10. Del Bianco, C., Aster, J.C. & Blacklow, S.C. Mutational and energetic studies of Notch 1 transcription complexes. *J Mol Biol* **376**, 131-40 (2008).