Electronic supplementary information

Macrocyclic Peptides that Inhibit Wnt Signaling via Interaction with Wnt3a

Manuel E. Otero-Ramirez, [a] Kyoko Matoba, [b] Emiko Mihara,[b] Toby Passioura, [ac] Junichi Takagi [b*] and Hiroaki Suga [a*]

[a] Department of Chemistry, Graduate School of Science, The University of Tokyo, Tokyo 113-8654, Japan

[b] Laboratory of Protein Synthesis and Expression, Institute for Protein Research, Osaka University, Osaka 565-0871, Japan

[c] Sydney Analytical, School of Chemistry and School of Life and Environmental Sciences, The University of Sydney, Sydney 2006, Australia

*To whom correspondence should be addressed: takagi@protein.osaka-u.ac.jp and hsuga@chem.s.u-tokyo.ac.jp

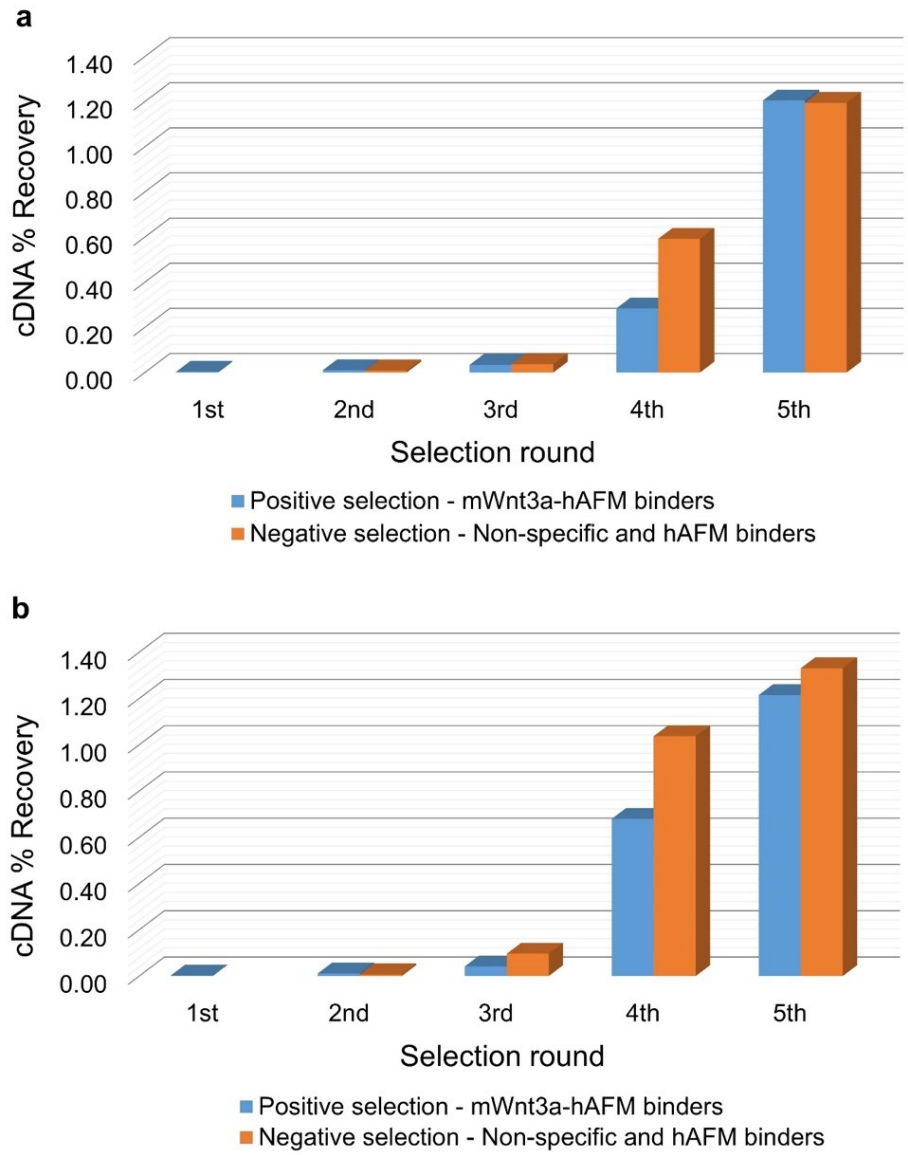

Figure S1 qPCR selection results of targeting the mWnt3a-hAFM complex with the RaPID system. (**a**) Graph shows recovered cDNA per round of selection against mWnt3a-hAFM, obtained from the macrocyclic peptide library initiated by D-Tyr. (**b**) Similar results corresponding to the library initiated by L-Tyr. Blue bars indicate the fraction corresponding to the cDNA of peptides bound to the complex, while orange bars indicate non-specific binders, including ones binding to only-hAFM-loaded magnetic beads.

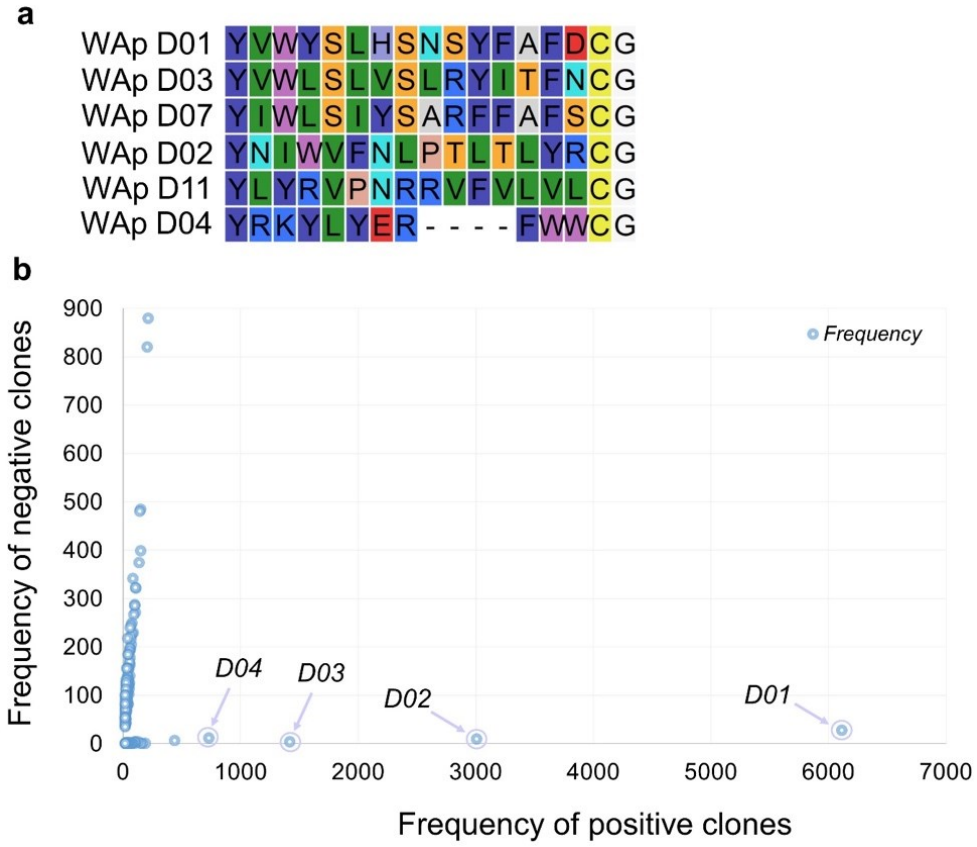

Figure S2 Alignment and 2D analysis of sequences in the enriched D-Tyr-initiated library. (**a**) Alignment of a few of the most enriched (frequent) sequences found within the library corresponding to the final round of selection against mWnt3a-hAFM. (**b**) 2D analysis of a set of the 80 most frequent sequences from said library, found to bind to hAFM (negative clones) or mWnt3a-hAFM (positive clones). Individual sequences are marked as blue spots, with some highlighted for comparison.

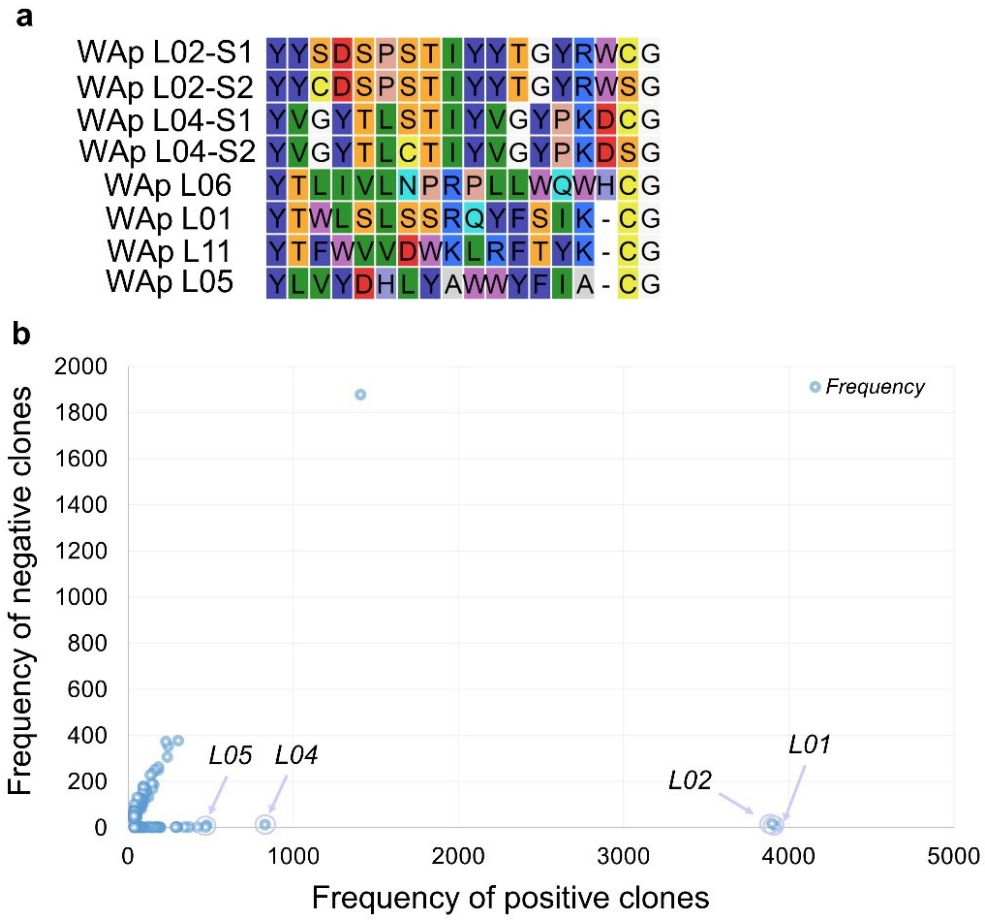

Figure S3 Alignment and 2D analysis of sequences in the enriched L-Tyr-initiated library. (**a**) Alignment of a few of the most enriched (frequent) sequences found within the library corresponding to the final round of selection against mWnt3a-hAFM. (**b**) 2D analysis of a set of the 80 most frequent sequences from said library, found to bind to hAFM (negative clones) or mWnt3a-hAFM (positive clones). Individual sequences are marked as blue spots, with some highlighted for comparison.

Peptide name	Sequence	Read number
WAp-D01	^{Ac}yVWYSLHSNSYFAFDCG	6114
WAp-D02	^{Ac}yNIWVFNLPTLTLYRCG	3010
WAp-D03	^{Ac}yVWLSLVSLRYITFNCG	1420
WAp-D04	^{Ac}yRKYLYERFWWCG	440
WAp-D07	^{Ac}yIWLSIYSARFFAFSCG	191
WAp-D11	^{Ac}yLYRVPNRRVFVLVLCG	150
WAp-L01	^{Ac}YTWLSLSSRQYFSIKCG	3924
WAp-L02-S1	^{Ac}YYSDSPSTIYYTGYRWCG	3899
WAp-L02-S2	^{Ac}YYCDSPSTIYYTGYRWSG	3899
WAp-L04-S1	^{Ac}YVGYTLSTIYVGYPKDCG	829
WAp-L04-S2	^{Ac}YVGYTLCTIYVGYPKDSG	829
WAp-L05	^{Ac}YLVYDHLYAWWYFIACG	477
WAp-L06	^{Ac}YTLIVLNPRPLLWQWHCG	471
WAp-L11	^{Ac}YTFWVVDWKLRFTYKCG	294

Table S1 Chosen sequences from libraries selected against mWnt3a-hAFM. Shown is an alignment of the 14 peptides chosen for clone assays and synthesis, originated from D-Tyr (WAp-D) and L-Tyr (WAp-L)-initiated libraries found to bind the mWnt3a-hAFM complex. Sequences are numbered in increasing order of frequency (number of reads) within each recovered library at round 5.

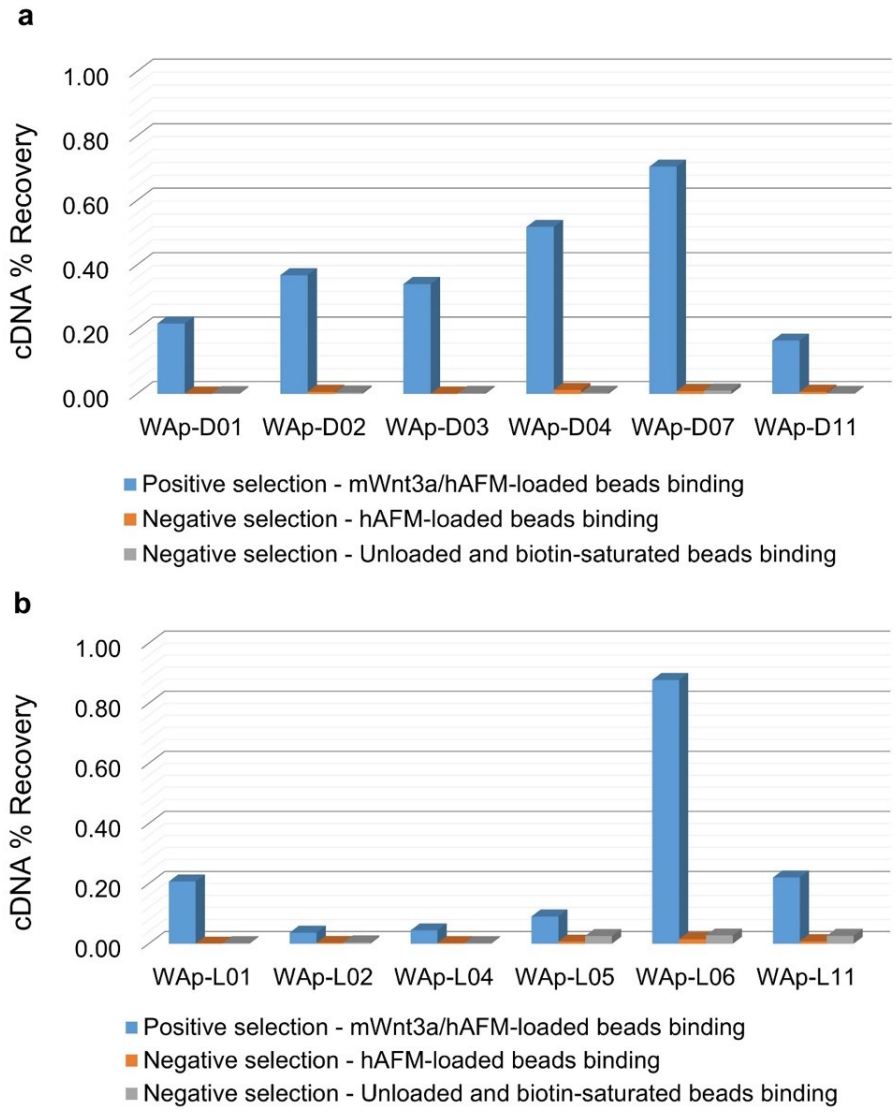

Figure S4 qPCR clone assay results of selected peptides against mWnt3a-hAFM. (**a**) Graph shows results obtained from single-clone, single-round selection assays (clone assays) targeting beads loaded with mWnt3a-hAFM (blue bars), hAFM (orange bars), or unloaded and biotin-saturated (gray bars) using individual sequences chosen from the selected libraries, all initiated by D-Tyr. (**b**) Similar results from the remaining sequences initiated by L-Tyr. Note that peptides WAp-L02 and WAp-L04 are shown as single peptides due to the simultaneous ribosomal expression of their S1 and S2 forms in the assay.

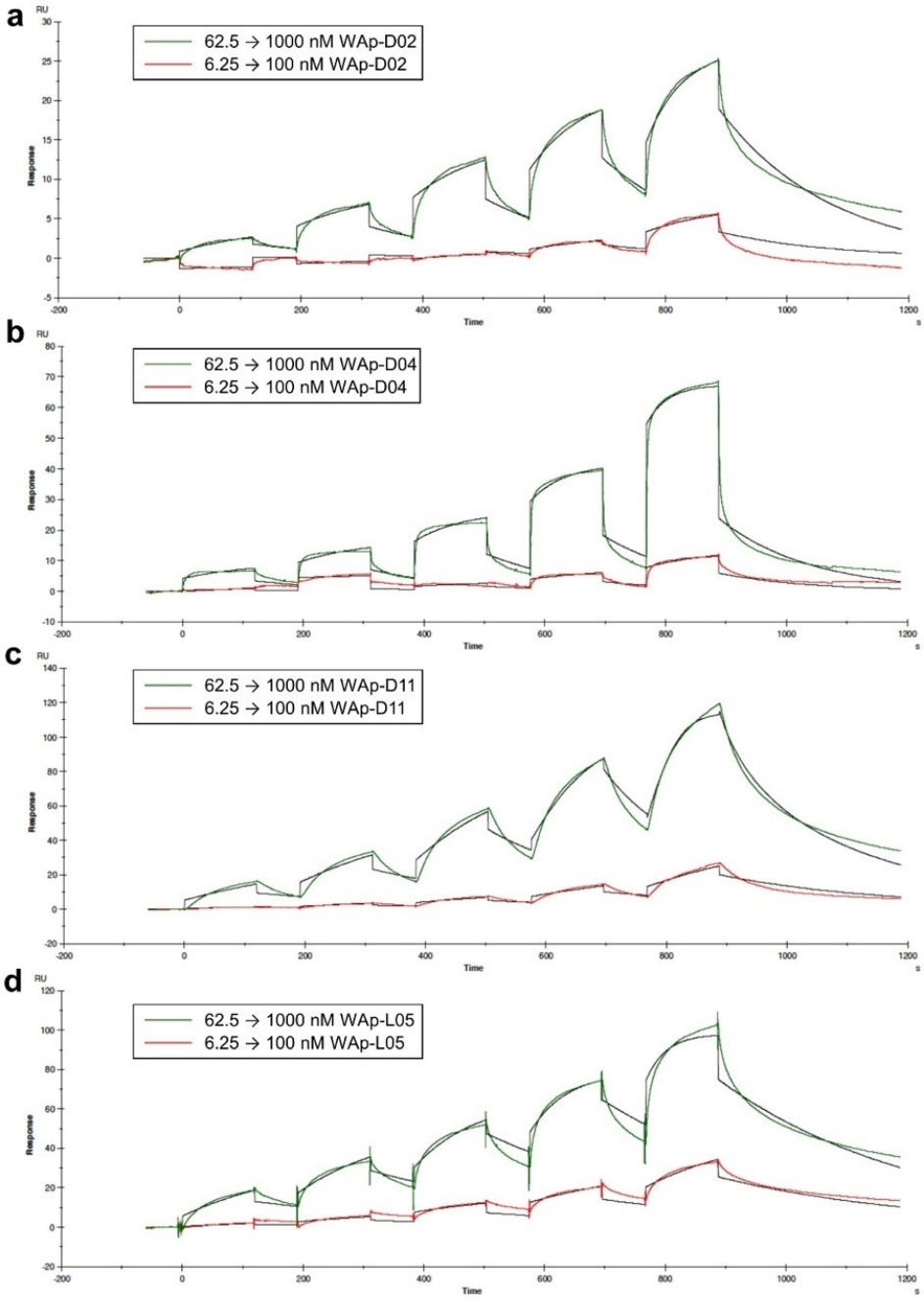

Figure S5 Binding profiles (kinetic analysis) of peptides selected against mWnt3a-hAFM. Sensorgrams show results from concentration ranges of 62.5 to 1000 nM (green) and 6.25 to 100 nM (red) of representative peptides WAp-D02 (**a**), WAp-D04 (**b**), WAp-D11 (**c**) and WAp-L05 (**d**), considered to show the most stable and reliable binding data. Black lines indicate software fitting for K_D value determination.

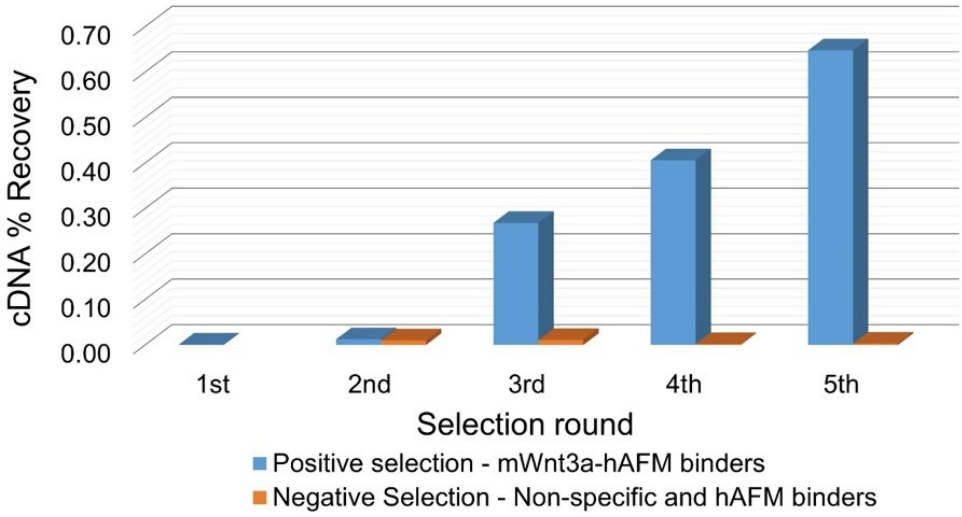

Figure S6 qPCR selection results of targeting the mWnt3a-hAFM complex with a WAp-D04-focused library through the RaPID system. Graph shows recovered cDNA per round of selection obtained from the focused macrocyclic peptide library, initiated by D-Tyr. Blue bars indicate the fraction corresponding to cDNA of peptides bound to the complex, while orange bars indicate non-specific binders, including ones binding to only-hAFM-loaded magnetic beads.

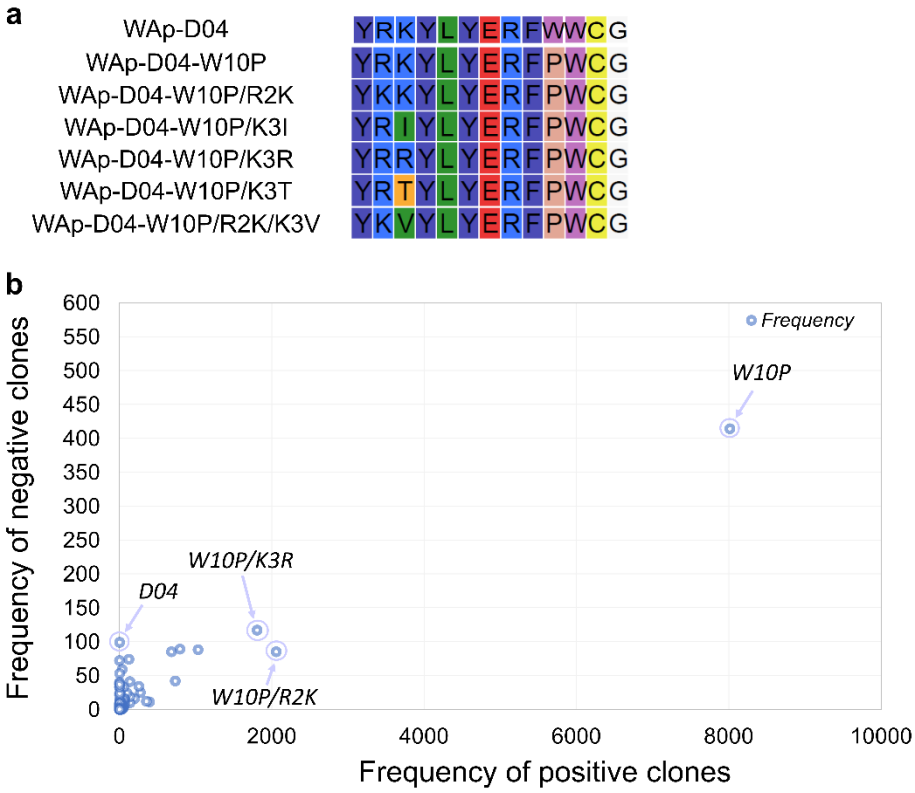

Figure S7 Alignment and 2D analysis of sequences of the enriched WAp-D04-focused library. (**a**) Alignment of a few of the most enriched (frequent) sequences found within the library corresponding to the final round of selection against mWnt3a-hAFM. (**b**) 2D analysis of a set of the 80 most frequent sequences from said library, found to bind to hAFM (negative clones) or mWnt3a-hAFM (positive clones). Individual sequences are marked as blue spots, with some highlighted for comparison. Additionally, original peptide WAp-D04 (D04 in graph) is also shown, displaying the presence of the parental sequence in the recovered focused library.

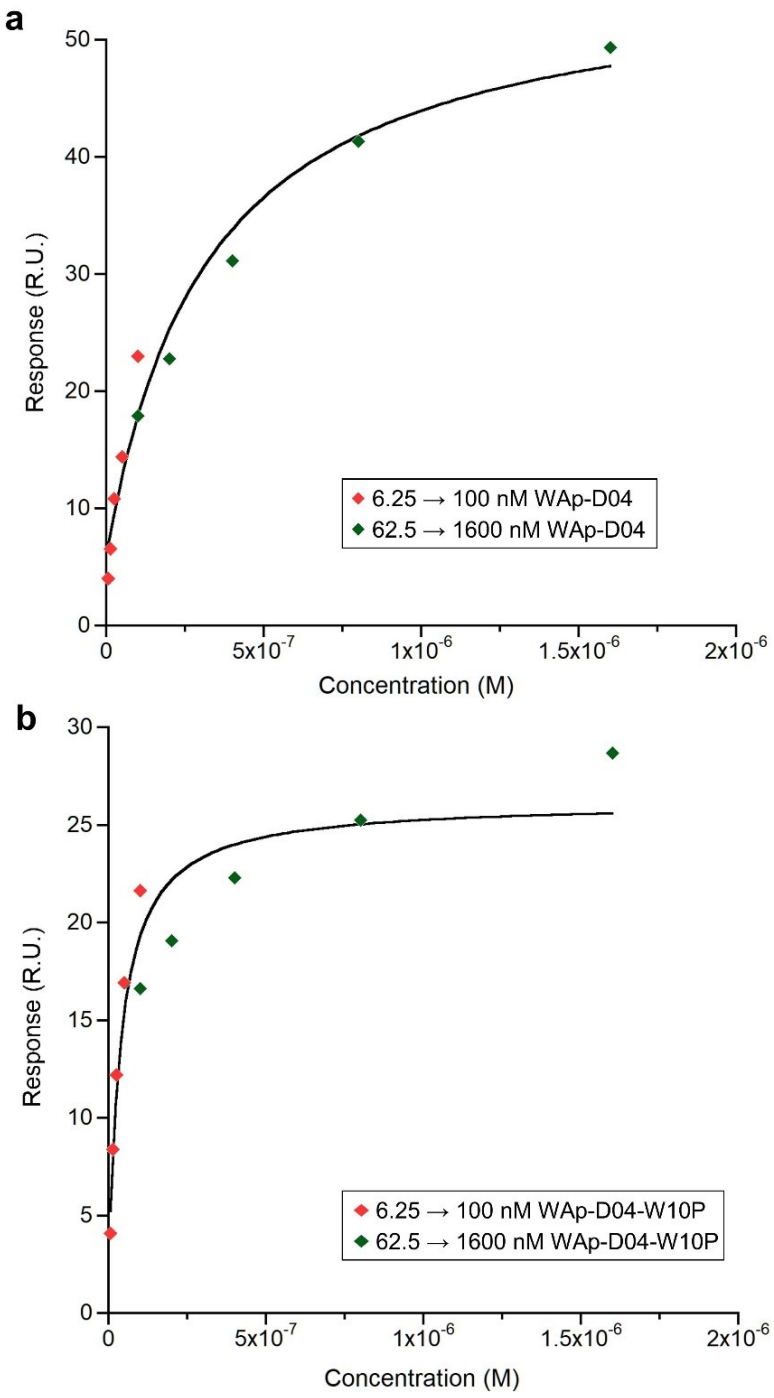

Figure S8 Steady state binding profiles of original (WAp-D04) and optimized (WAp-D04-W10P) peptide inhibitors of Wnt signaling found in this work. (**a**) Sensorgram corresponding to concentration ranges of 6.25 to 100 nM (red diamonds) and 100 to 1600 nM (green diamonds) of the parental peptide inhibitor WAp-D04. (**b**) Similar analysis for optimized peptide WAp-D04-W10P. Black curves indicate software fitting for K_D value determination.