## **Supporting Information**

## Variable autoinhibition among deafness-associated variants of Diaphanous 1 (DIAPH1)

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**Figure S1. Varied auto inhibition of DIAPH1-FFC mutants by INF2-NT.** Pyrene-actin assembly assays were carried out with 2  $\mu$ M actin (5% pyrene-labeled), ± 5 nM of FFC, either (A) WT; (B) *ttaa* ;(C) 1213x; (D)  $\Delta ag$ ; or (E) M1199D, with varied concentration of INF2-NT. Concentrations of NT are indicated in (A), and the color scheme is the same in panels (B-E), with actin plus 200 nM NT and no FFC in gray. (F) Inhibition curves were calculated from the slopes of raw pyrene traces at 100 s and fit with a quadratic binding model that assumes 1:1 binding between dimers.

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Figure S2. Varied auto inhibition of DIAPH1-FFC mutants. *Replicate of Figure 4 with different protein preparations*. Pyrene-actin assembly assays were carried out with 2  $\mu$ M actin (5% pyrene-labeled), ± 5 nM of FFC, either (A) WT; (B) *ttaa* ;(C) 1213x; (D)  $\Delta ag$ ; or (E) M1199D, with varied concentration of WT DIAPH1-NT. Concentrations of NT are indicated in (A), and the color scheme is the same in panels (B-E), with actin plus 200 nM NT and no FFC in gray. (F) Inhibition curves were calculated from the slopes of raw pyrene traces at 100 s and fit with a quadratic binding model that assumes 1:1 binding between dimers.



Figure S3. Auto inhibition by DIAPH1-NT mutants. *Replicate of data in Figure 5 with different protein preparations.* Pyrene-actin assembly assays were carried out with 2  $\mu$ M actin (5% pyrene-labeled), ± 5 nM of WT-FFC, titrated with either (A) WT; (B) A265S; or (C) I530S DIAPH1-NT. Concentrations of NT are indicated in (A), and the color scheme is the same in panels (B,C), with actin plus 200 nM NT (no FFC) in gray. (D) Inhibition curves were calculated from the slopes of raw pyrene traces at 100 s and fit with a quadratic binding model that assumes 1:1 binding between dimers.



**Figure S4. Differential effects of cysteine-labeling on profilin isoforms.** Total internal reflection fluorescence (TIRF) microscopy of 1  $\mu$ M rabbit skeletal muscle actin (15% Cy3B-labeled on Cys374), 2 nM formin-FFC (*Drosophila* Cappuccino), and 2.5  $\mu$ M profilin: (A) human profilin1, (B) *Drosophila* profilin (chickadee), (C) *S. pombe* profilin. Each panel shows a snapshot from a TIRF movie at 10-20 minutes after the addition of polymerization buffer. Slow-growing filaments are indicated by pink arrows, and are presumed not to be formin-bound. In the presence of *S. pombe* profilin, the formin-elongated filaments (unmarked) are of similar brightness to the slow-growing filaments. Actin was labeled with Cy3B-maleimide as described Chen et al.<sup>1</sup> Cappuccino-FFC was purified and TIRF experiments were carried out as in Vizcarra et al.<sup>2</sup> Profilin isoforms were purified as in Bor et al.<sup>3</sup>

Table S1. Sequences for proteins used in this study.		
Name	BC117257 numbering	amino acid sequence
	2011/10/ 10/10/10	
DIAPH1-NT	1 - 548	DPLOGMMEPGGSLGFGRGTROKKKGSPDELPSAGGDGKSKKFLERTSMRIKKEKENSAHROSSASVGDDFTAGSLQDVSDEQVLUFE QMLLDMNLNEEKQQFLREKDIIKREMVSQYLYTSKAGMSQKESKSAMMYIQELRSGLRDMFLSCLESLRVSLNNPVSWVQTFGAEGLAS LLDILKRLHDEKETAGSVDSRNHEIIKCLKAFMNNFGIKTMLETEGILLLVRAMDPAVFNMMIDAAKLISALCILPQFEDNMERVLEAM TERAENDEVERPQFLLDGLKSGTTIALKVGCLQIINALITPAEELDFRVHIRSELMRLGHQVLQDLERIENEDMEVQLNVFDEQGEEDSVDL KGRLDDIRMENDFWEVFGLULTNVKDSKAEPHFEISIQHLLVRNVDERAFVYKLIEGISJULHKNGADPDFKCHLQIEGLIDQMI DKKKVEKSEAKAAELEKKLDSELTARHELQVEMKKMESDFEQKLQDLQGEKDALHSEKQQIATEKQDLEAEVSQLTGEVAKLTKELED*
DIAPH1-NT I530S	1 – 548 (15215)	GULGSMEPFOSLEFGKGIKUKKKGSPIELPSAGEDUGKSKKFLEKTISMKIKKEKEFNAAHKNISASITUDPIAQSLEQVILIPE QMLDMNINERQQPLERKDI IRKENVSQUITISKAGMSQKESISKAMUJIQELRSGLEMPHLISCLESIKVSLMNPVSWQTFGAGGLAS LLDILKRLHDEKEETAGSYDSRNKHEIIRCLKAFMNNKFGIKTMLETEEGILLLVRAMDPAVPNMMIDAAKLLSALCILPQPEDMNERVLEAM TERAEMDEVERQPLLOGLKSGTITALKVGCLQIINALITPAEELDFKVHIRSELMKLGLHQVLQDLEEINEDMENVQLNVFDEQGEEDSYDL KGRLDDIRMEMDDFWEVFGILUNTVKDSRABPHFSIIQHLLVRNVDERAFQYYKLIEECISQIVLHKNGADPEVFCRHLQIEGSLIQMI DKTKVEKSEAKAAELEKKLDSELTARHELQVEMKKMESDFEQKLQDLQGEKDALHSEKQQ <u>S</u> ATEKQDLEAEVSQLTGEVAKLTKELED*
DIAPH1-NT A265D	1 – 548 (A256D)	GPLGSMEPFOGSLOFGGSLOFGRGTRDKKKGSPDELPSAGGDGGKSKKFLERFTSMRIKKEKERNSAHRNSSAYGDDF7AQSLQDVSDEQVLUFE QMLDMNINERQQPLERKDI IRKENVGYLITTSKAMGVESSKSAMVIJQELRSGLENDPLISCLESLRVSLNNPVSWQTFGAGGLAS LLDILKRLHDEKEETAGSYDSRNKHEIIRCLKAFMNNKFGIKTMLETEEGILLLVRAMDPAVPNMTIDAAKLLS TERABMDEVERPQFLLGGLKSGTTIALKVGCLQLINALITPAEELDFRVHIRSELMRLGLHQVLQDLREIENEDMRVQLNVFDEQGEEDSYDL KGRLDDIRMENDFWEVFGILLNTVKDSKAEPHFLSILQHLLVRNVTARPQYYKLIECISQIVLHRNGADPBYCRHLDEIEGILOGMI DKTKVEKSEAKAAELEKKLDSELTARHELQVEMKKMESDFEQKLQDLQGEKDALHSEKQQIATEKQDLEAEVSQLTGEVAKLTKELED*
DIAPH1-NT A265S	1 – 548 (A256S)	GULGSMEPFOSLEFKGTKUKKKSSPLEIPSAGDUGKSKKFLEKTISMIIKEREFISMIIKEREFISTIODPTAGSLUPVSDEQUFULFE GWLDMNINERCQPIERENIIIKERWSQUITISKAMSQKSSKSAMUTJQELRSGLEMPLISCLESLRVSLANNPVSWQTFGAGCLAS LLDILKRLHDEKEETAGSYDSRNKHEIIRCLKAFMNNKFGIKTMLETEEGILLLVRAMDPAVPNMTIDAAKLLS <u>S</u> LCILPQPEDMNERVLEAM TERARMDEVERQPLLOGLKSGTTIALKVGCLQLINALITPAEELDFRVHIRSELMKLGLHQVLQDLREIENDEMRVQLWVFDEQGEDSYDL KGRLDDIRMEMDDFRWFQILUNTVKDSRAPHFISILQHLLVRNVDERPGYVKIIESCISQIVLHKNGADPERCKHLQIEIGLIDAMI DKTKVEKSEAKAAELEKKLDSELTARHELQVEMKKMESDFEQKLQDLQGEKDALHSEKQQIATEKQDLEAEVSQLTGEVAKLTKELED*
INF2-NT	1 – 420 (NP_071934.3)	CPLSGMSVKSGAQRKWAALKEKLOFQDSDPTEANLESADPELCIRLIQMFSVVNYSGLRKRLEGSDGGWWQFLEQSGLDLLLEALARLSGRGVA RISDALIQLCVSCURAWNSGGIEYILSANGGVAGLAGALDTSNVVVKKQVFELLAALCISPEGHVLTLDALDHXKVCSGQVPRFSIVMUEL SGSDNVPYVVTLLSVINAVILGPEDLRARTQLRNEFIGLQLLDVLARLRDLEDADLLIQLEAFEEAKAEDEEELLRVSGGVDMSSHQEVFASLFH KVSCSPVSAQLLSVLQGLHLEPTLRSSQLIWEAELSJVNRAVLLASDAQECTLEEVVERLLSVKGRPRPSPLVKAHKSVQANLDQSQRGSSPQN TITPRFSVEGQOPAAAACEVDUAGSSIKVSQPRALEQQAST
DIAPH1-FFC	<b>549 – 1262</b> his and strep tags not cleaved	MGSSHHHHHMGSAKKEMASLSAAAITVPFSVPSRAPVPPAPPLPGDSGTIIPPPPAPGDSTTPPPPPPPPPPPPPPPPGGCSISSPSLPGGTAI SPPPPLSGDATIPPPPLPEGVGIPSPSSLPGGTAIPPPPPLPGSARIPPPPPPLPGSAGIPPPPPPLGEAGMPPPPPPLGGPGIPPPPF GGPGIPPPPGMGMPPPPPFGGVPAAPVLPGLTPRKLTKEEVQLRRRMSKLVAEDLSQDCFWTKVKBDRENNELFAKLTLTFSATTKT SKAKKDQEGEEKSVQKKVKUELKVLDSKTAQNLSIFLGSFFMPYQEINVILEVNEAVITESMIQNLIKQMEPEPDLAKLSELKDEYDDLA ESSQFGVMGTVPRLRPRLMAILEKLQFSEQVENIKPEIVSVTAACELRKSESFSNLLEITLIUGMYMMAGSRMAGAFGFNISFLCKLRDTK STDQKMTLLHFLAELCENDYFDVLKFPDELHAVEKASRVSAENLQKKLDQMKKQISDVERDVQNFFAATBLKKKTSVKMSEFVKDAQEQYNKL RMMHSNMETJYKELGEYFFFDFKLSVEEFFMDLIMFNMFLQAVKENQKRKETESKMTRAKLAKEKAEKRELEKQCKREQLIMMAGGEG VMDSLLEALQSGAAFRRKRGPRQANRKAGCAVTSLLASELTKDDAMAAVFAKVSKNSETFFTILEEAKELVGRAS <u>VMPRGSWSHPQFEK*</u>
DIAPH1-FFC ttaa	<b>549 – 1232</b> his and strep tags not cleaved	MGSS.HHHHHMGSAKKEMASLSAAAITVPPSVPSRAPVPPAPPLPGDSGTIIPPPPAPGDSTTPPPPPPPPPPPPPPLPGGVCISSPPSLPGGTAI SPPPELGGDATIPPPPPLEGGVGIPSSLPGGTAIPPPPLEGSARIPPPPPLGGAGIPPPPPLEGGAGHPPPPLEGGPGIPPPPPP PGGGDIPPPPGGWGMPPPPPPFPFGGVPAAPULPFGIPRKLYKEPQLRRNWSKUVAEDLSGDGVMTKVKEDREPNBLFAKLTIFSAQTKT SKAKKDQEGGEKKSVQKKKVELKVLDSKTAQNLSIFLGSFMMYQDINVLEVVLEAVLTESMIQNLKKMEDEGLAMAPPPPLLGGGGIPPPPPL SKAKKDQEGGEKKSVQKKKVELKVLDSKTAQNLSIFLGSFMMYQDINVLEVVLEAVLTESMIQNLKMARAGAFGNISTLCKLRDTK SKAKKDQEGGEKKSVQKKKVELKVLDSKTAQNLSIFLGSFMMYQDINVLEVVLESVLGSVTULVMNAGSNAGAFGNISTLCKLRDTK STAKKNDEGGEKKSVQKKKVELKVPDLALAVEKSVSAENLGKNLDQMKKQISDVENDVQNFPAATDEKDKFVEKMTSFVKDAQEQVNKL RMMISNMETIVELGEVELPPKLSVEEFFMDLIMFNMMELGAVENGKREATEEKNRARALKAKEKAEKERLEKQQKREQLIDMNAEGDETG VMDSLLEALQSGAAFRRKRGPRQVNQQEGRVCSHISASFGADQG <u>LVPBGSWSHPQFEK</u> *
DIAPH1-FFC 1213x	<b>549 – 1202</b> his and strep tags not cleaved	MGSS_MHMHMIGGAKKENASLSAAAITVPESVPSRAPVPFAPLPCDSGTIIPPPFACDGSTTPPPPPPPPPPPPPPPLCGVCISSPESLPGGTAI SPPPPLGSGATITPPPPLEGGUGTBSSELFGGTAITPPPPPPLGGARITPPPPPPLGGAGTPPPPPPLGGAGCISPPPPF PGGPGIPPPPGMGMPPPPPGFGVPAAFVLPFGLTPKKLYKPEVQLRRPNWSKLVAEDLSQDCFWTKVKEDRFENNELFAKLTLTFSAQTKT SKAKKDQEGGEKKSVQKKKVKELKVLDSKTAQNLSIFLGSFMPYQDINN'ILEVNEAVITESMIQNIKQMEPEPGLKNLSELKDEYDDLA ESEQGEVMVTVFLRFRALITLFKLGFSEQVENIKPEIVSVTAACEELKEKSESFSNLEITLUVGNYMNAGSNAGAFGNISTCLKLDRY STDQKMTLHFLAELCENDYPDVLKFPDELAHVEKASRVSAENLQKNLDQMKKQISDVERDVQNFPAATDEKDKFVEKMTSFVKDAQEQYNKL RMMHSNMETIVKELGSVEJPDKLSVEFFDDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKAEKERLEKQQKREQLIDMNAEGDETG VMDSLLEALQSGAAF LVPRGSWSHPQFEK*
DIAPH1-FFC <b>Δ</b> σg	<b>549 – 1229</b> his and strep tags not cleaved	MGSSMINNINGSAKKENASLSAAAITVPFSVPSKAPVPPAPLPGDSGTIIPPPAPGGGSTTPPPPPPPPPPPPPPPPPPPPPPPPPPPPP
DIAPH1-FFC M1199D	<b>549 – 1262 (M1189D)</b> his and strep tags not cleaved	MGSSMINNINGSAKKENASLSAAAITVPFSVPSKAPVPPAPLPGDSGTIIPPPPAGOSTTPPPPPPPPPPPAGGVCISSPPSLPGGTAI SPPPEJGSDGTIPPPPEJGGGVGIPSPSLPGGTAIPPPPPLGSARIPPPPPLGSACHIPPPPPLGGAGVCISSPPSLPGGTAI SSPPEJGGDGEKKSVQKKVKELKVLDSKTAQNLSIFLGSFRPVQEINVILEVNEAVLEDLSQDCFWTKVKEBRTENNELFAKLTLTFSATTKT SKAKKDQGGEGEKKSVQKKVKELKVLDSKTAQNLSIFLGSFRPVQEINVILEVNEAVLETSSIQNLIKGSRTEPPEPPLGKAUSELKDEYDDL ESEQFGVVMGTVPELRPRLNAILFKLQFSEQVENIKPEIVSVTAACEELKKSESFSNLLEITLLVGYNMAGSNAGAFGFNISFLCKLRDTK STOQKWTLUFLFALCCENDYDVLKFPDELAHVEKASKVSAENLOKNLDQMKKQISDVERDVQNFPAATDEEKCFVEKMTSFVKDAQQVIKL RMMHSNMETLYKELGEYFLFDFKKLSVEEFFMDLHNFRNMFLQAVKENQKRRETEEKMRRAKLAKEKAEKERLEKQQKREQLIDMNAEGDETG VDSLLEALQSGAAFRRKRGPRQANRKAGCAVTSLLASELTKDDAMAAVFAKVSKNSETFFTILEEAKELVGRAS <u>VPRGSWSHPQTEK*</u>
KEY: Red= Start codon or sequence left from protease cleavage Orange= 6X his-tag Green= Thrombin cleavage site Purple= Streptavidin Blue = point mutation *= stop codon		

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## References

- Chen, C. K., Sawaya, M. R., Phillips, M. L., Reisler, E. & Quinlan, M. E. Multiple Forms of Spire-Actin Complexes and their Functional Consequences. *J. Biol. Chem.* 2012, 287 (13), 10684. https://doi.org/10.1074/jbc.M111.317792
- (2) Vizcarra, C. L., Bor, B. & Quinlan, M. E. The Role of Formin Tails in Actin Nucleation, Processive Elongation, and Filament Bundling. *J. Biol. Chem.* 2014, 289 (44), 30602. https://doi.org/10.1074/jbc.M114.588368
- (3) Bor, B., Vizcarra, C. L., Phillips, M. L. & Quinlan, M. E. Autoinhibition of the formin Cappuccino in the absence of canonical autoinhibitory domains. *Mol. Biol. Cell* **2012**, 23 (19), 3801. https://doi.org/10.1091/mbc.e12-04-0288

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