SUPPORTING INFORMATION

In-vivo crystals reveal critical features of the interaction between CFTR and the PDZ2 domain of Na^+/H^+ exchange cofactor NHERF1

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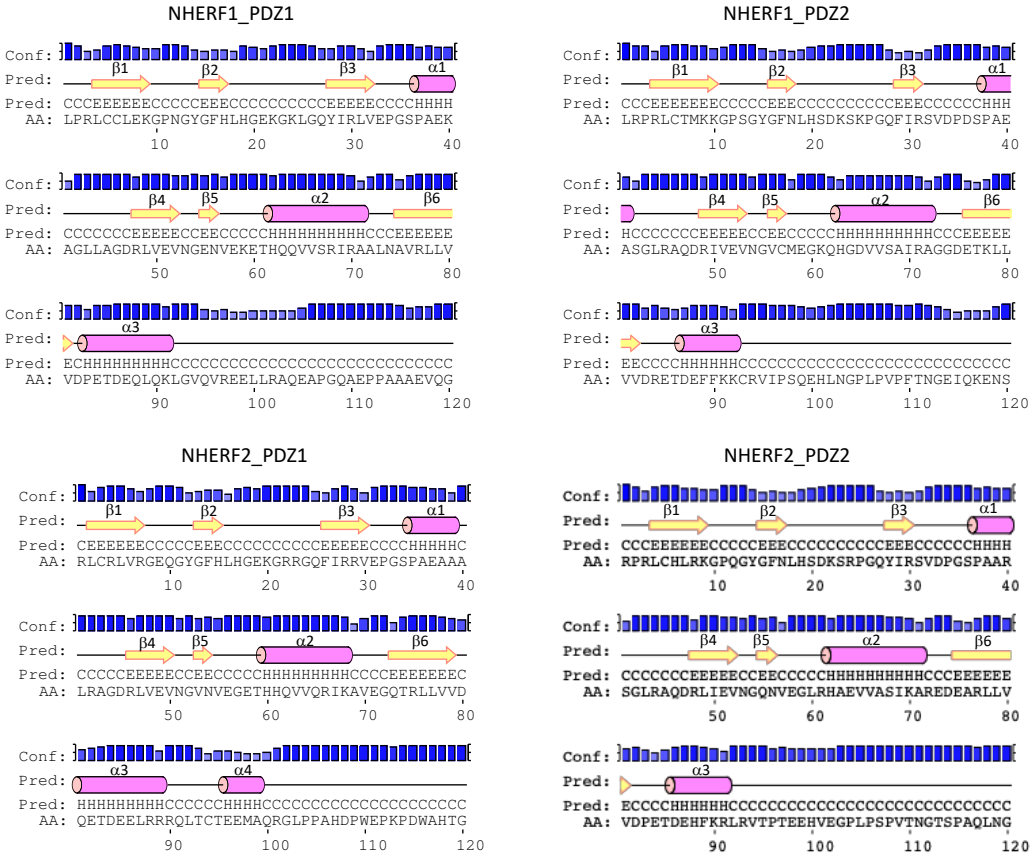
Supporting information

Figure S1. Overlay of extended and canonical NHERF1 PDZ2 domain. Extended PDZ2 domain of NHERF1 obtained in this study (green) is overlaid with the crystal structure of the canonical domain (PDB: 2OZF) (orange).



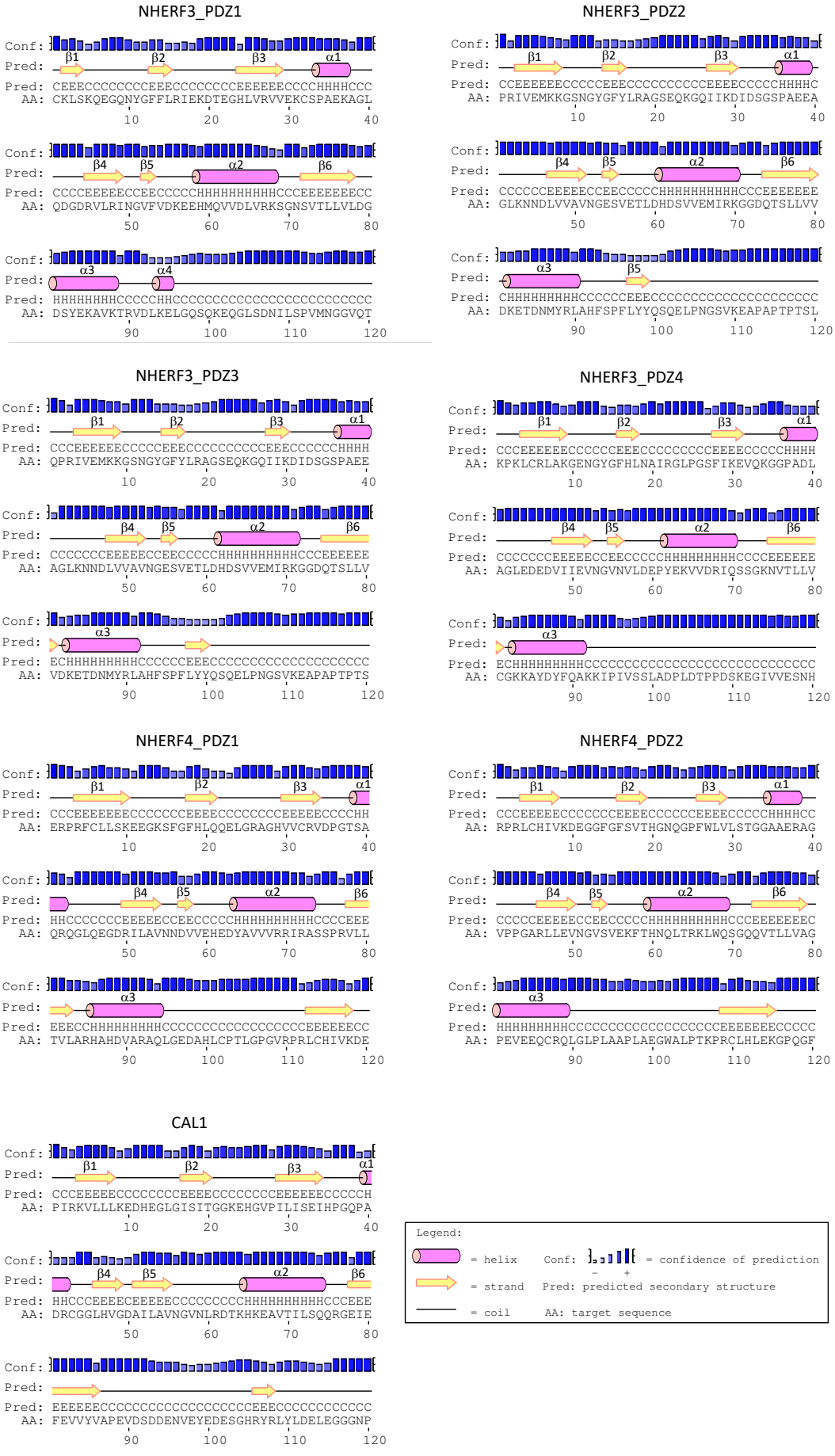

NHERF3_PDZ1
Conf:
Pred:
β1 β2 β3 α1
Pred: CEEECCCCCCCCCEEECCCCCCCCCEEEEEECCCCHHHHCCC
AA: CKLSKQEGQNYGFFLRIEKDTEGHLVRVVEKCSPAEKAGL
10 20 30 40
β4 β5 α2 β6
Pred: CCCCEEEEECCEECCCCCHHHHHHHHHHCCCEEEEEEECC
AA: QDGDRVLRINGVFVDKEEHMQVVDLVRKSGNSVTLLVLDG
50 60 70 80
α3 α4
Pred: HHHHHHHHCCCCCHHCCCCCCCCCCCCCCCCCCCCCCCCC
AA: DSYEKAVKTRVDLKELGQSQKEQGLSDNILSPVMNGGVQT
90 100 110 120
NHERF3_PDZ2
Conf:
Pred:
β1 β2 β3 α1
Pred: CCEEEEEECCCCCEEECCCCCCCCCCEEEECCCCCCHHHHC
AA: PRIVEMKKGSNGYGFYLRAGSEQKGQIIKDIDSGSPAEEA
10 20 30 40
β4 β5 α2 β6
Pred: CCCCCCEEEEECCEECCCCCHHHHHHHHHHCCCEEEEEEE
AA: GLKNNDLVVAVNGESVETLDHDSVVEMIRKGGDQTSLLVV
50 60 70 80
α3 β5
Pred: CHHHHHHHHHCCCCCCEEECCCCCCCCCCCCCCCCCCCCC
AA: DKETDNMYRLAHFSPFLYYQSQELPNGSVKEAPAPTPTSL
90 100 110 120
NHERF3_PDZ3
Conf:
Pred:
β1 β2 β3 α1
Pred: CCCEEEEEECCCCCEEECCCCCCCCCCEEECCCCCCHHHH
AA: QPRIVEMKKGSNGYGFYLRAGSEQKGQIIKDIDSGSPAEE
10 20 30 40
β4 β5 α2 β6
Pred: CCCCCCCEEEEECCEECCCCCHHHHHHHHHCCCEEEEEEE
AA: AGLKNNDLVVAVNGESVETLDHDSVVEMIRKGGDQTSLLV
50 60 70 80
α3
Pred: ECHHHHHHHHHCCCCCCEEECCCCCCCCCCCCCCCCCCCC
AA: VDKETDNMYRLAHFSPFLYYQSQELPNGSVKEAPAPTPTS
90 100 110 120
NHERF3_PDZ4
Conf:
Pred:
β1 β2 β3 α1
Pred: CCCEEEEEECCCCCCEEECCCCCCCCCEEEECCCCCCHHHH
AA: KPKLCRLAKGENGYGFHLNAIRGLPGSFIKEVQKGGPADL
10 20 30 40
β4 β5 α2 β6
Pred: CCCCCCCEEEEECCEECCCCCHHHHHHHHHCCCCEEEEEE
AA: AGLEDEDVIIEVNGVNVLDEPYEKVVDRIQSSGKNVTLLV
50 60 70 80
α3
Pred: ECHHHHHHHHHCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
AA: CGKKAYDYFQAKKIPIVSSLADPLDTPPDSKEGIVVESNH
90 100 110 120
NHERF4_PDZ1
Conf:
Pred:
β1 β2 β3 α1
Pred: CCCEEEEEEECCCCCCCEEEECCCCCCCCEEEEECCCCHH
AA: ERPRFCLLSKEEGKSFGFHLQQELGRAGHVVCRVDPGTSA
10 20 30 40
β4 β5 α2 β6
Pred: HHCCCCCCCEEEEECCEECCCCCHHHHHHHHHHCCCCEEE
AA: QRQGLQEGDRILAVNNDVVEHEDYAVVVRRIRASSPRVLL
50 60 70 80
α3
Pred: EEECCHHHHHHHHHCCCCCCCCCCCCCCCCCCCEEEEECC
AA: TVLARHAHDVARAQLGEDAHLCPTLGPGVRPRLCHIVKDE
90 100 110 120
NHERF4_PDZ2
Conf:
Pred:
β1 β2 β3 α1
Pred: CCCEEEEECCCCCCCCEEEECCCCCCCEEEECCCCCHHHHCC
AA: RPRLCHIVKDEGGFGFSVTHGNQGPFWLVLSTGGAAERAG
10 20 30 40
β4 β5 α2 β6
Pred: CCCCCEEEEECCEECCCCCHHHHHHHHHHCCCEEEEEEEC
AA: VPPGARLLEVNGVSVEKFTHNQLTRKLWQSGQQVTLLVAG
50 60 70 80
α3
Pred: HHHHHHHHHCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
AA: PEVEEQCRQLGLPLAAPLAEGWALPTKPRCLHLEKGPQGF
90 100 110 120
CAL1
Conf:
Pred:
β1 β2 β3 α1
Pred: CCCEEEEECCCCCCCCEEEECCCCCCCCCEEEEEECCCCCH
AA: PIRKVLLLKEDHEGLGISITGGKEHGVPILISEIHPGQPA
10 20 30 40
β4 β5 α2 β6
Pred: HHCCCEEEECEEEEECCCCCCCCCHHHHHHHHHHCCCEEE
AA: DRCGGLHVGDAILAVNGVNLRDTKHKEAVTILSQQRGEIE
50 60 70 80
Pred: EEEEEECCCCCCCCCCCCCCCCCCCEEECCCCCCCCCCCC
AA: FEVVYVAPEVDSDDENVEYEDESGHRYRLYLDELEGGGNP
90 100 110 120
Legend:
= helix
= strand
= coil
Conf: = confidence of prediction
Pred: predicted secondary structure
AA: target sequence

Figure S2. Secondary structure prediction for the PDZ domains of the NHERF family. A third helix (α3) after the β6 strand is predicted for several PDZ domains within the NHERF family, but not for those outside such as CAL1. Note: A fourth helix (α4) is predicted only for NHERF2 PDZ1 and NHERF3 PDZ1, but is not predicted to be present in either NHERF1 PDZ1 or PDZ2. Secondary structure predictions were carried out using the PSIPRED server v3.3 (available at http://bioinf.cs.ucl.ac.uk/psipred) using the default settings.

```
Human       ISPSDRVKLFP--HRNSSKCKSKPQIA----ALKEETEEEVQDTRL 1480
Frog        --HSDRLKLFPLHRRNSSKRKSRPQIS----ALQEETEEEVQDTRL 1485
Rat         LSSSEKMKLFH--GRHSSKQKPRTQIT----AVKEETEEEVQETRL 1476
Mouse       ISSSEKMRFFQ--GRHSSKHKPRTQIT----ALKEETEEEVQETRL 1476
Zebrafish   ----ERLKLFP--RRNSSMRTPQSKLSSVTQTLQEEAEDNIQDTRL 1485
Dog         IGPPERPGLLP--HRLSSRQRSPSRIA----ALKEETEDEVQDTRL 1483
Horse       ISSSDPLKLFP--HRNSSKHKSRSKIA----ALQEETEEEVQETRL 1481
Bovine      ISPADRLKLLP--HRNSSRQRSRSNIA----ALKEETEEEVQETKL 1481
Rabbit      ISSSDRAKLFP--HRNSSKHKSRPQIT----ALKEEAEEEVQGTRL 1476
Sheep       ISPADRLKLLP--HRNSSRQRSRANIA----ALKEETEEEVQETKL 1481
                :   ::    * **       .::    :::**:*:::* *:*
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Figure S3. Cross-species sequence alignment of the CFTR C-terminus. Last 40 residues of CFTR were aligned using ClustalW2 with the default settings.

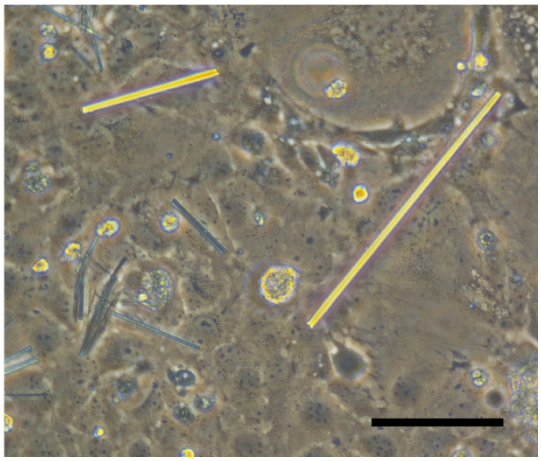


Figure S4. *In-cellulo* crystals formed by iBox-PDZ2-PAK4cat fusion construct. Crystals were imaged four days post-transfection in COS7 cells. Scale bar indicates 100 µm. Note: The different colors of the crystals likely results from the plastic of the petri dish which produces some polarization in the light which interacts with the lattices in the crystals. The colour of individual crystals therefore depends on their size and relative orientation in the field of view.

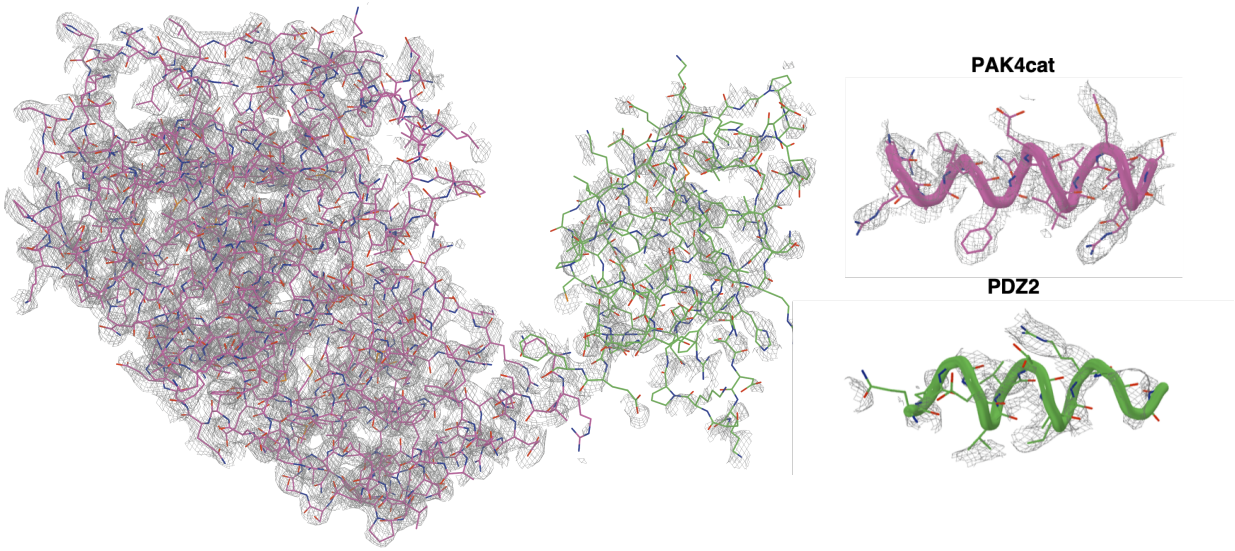


Figure S5. Unrefined electron density map obtained for the iBox-PDZ2-PAK4cat fusion construct. OMIT mesh map is contoured at 1.0 sigma and within 1.5 Å of the respective atoms. Left, iBox-PAK4cat is shown in magenta and PDZ2 in green. Right, comparison of electron density observed for typical helices in PAK4cat and PDZ.

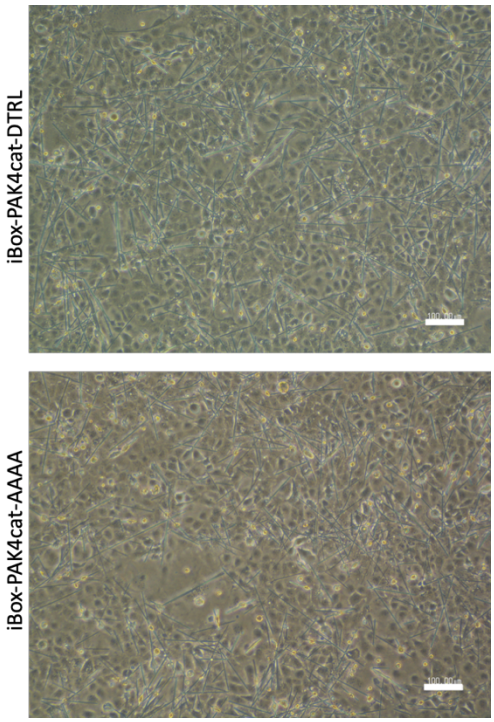
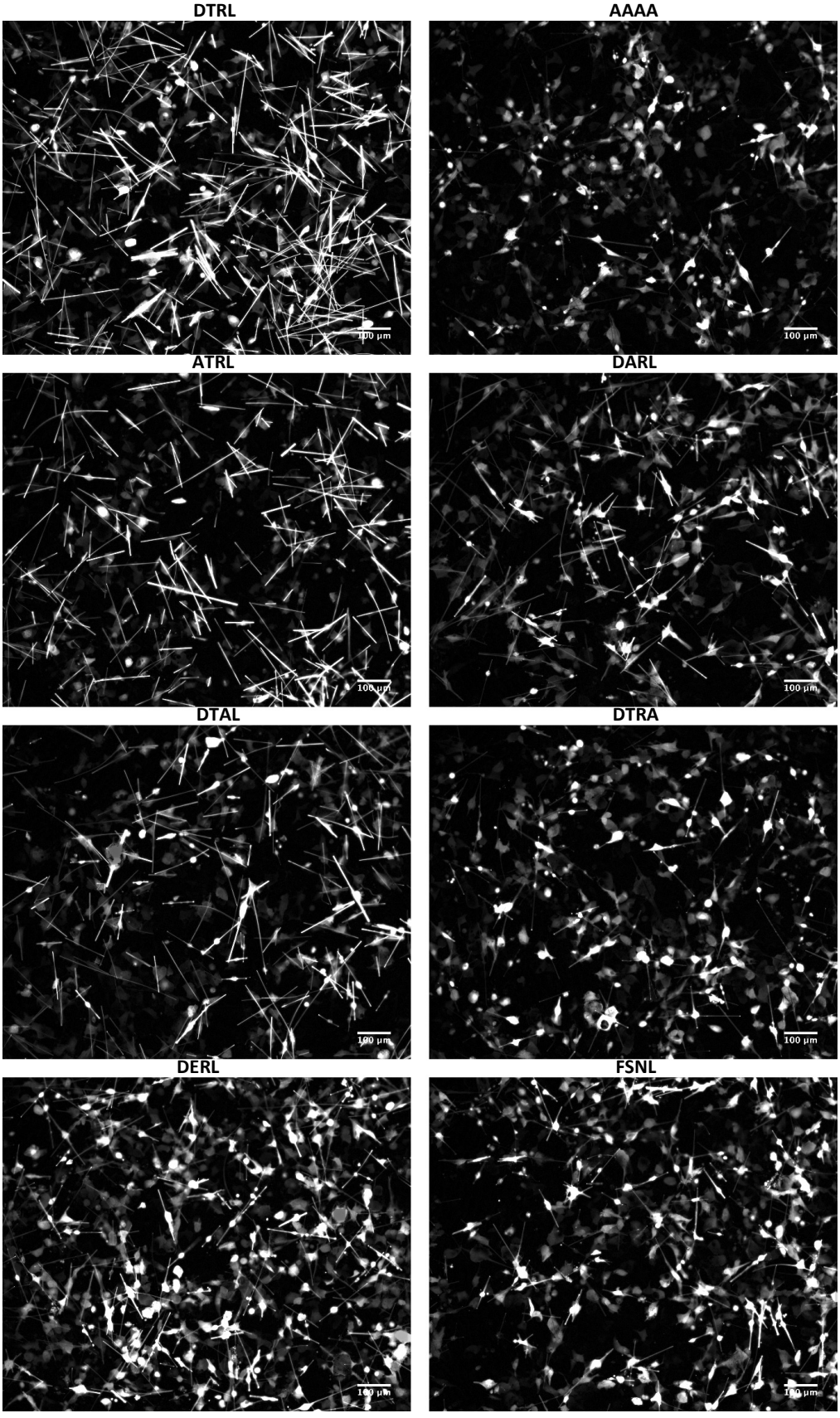


Figure S6. Light images of COS-7 cells co-transfected with GFP-PDZ1 and either iBox-PAK4cat-DTRL or iBox-PAK4cat-AAAA. Both iBox-PAK4cat construct variants appear to be similar in terms of crystal generation proficiency. Scale bar indicates 100 µm.

GFP-PDZ1



GFP-PDZ2

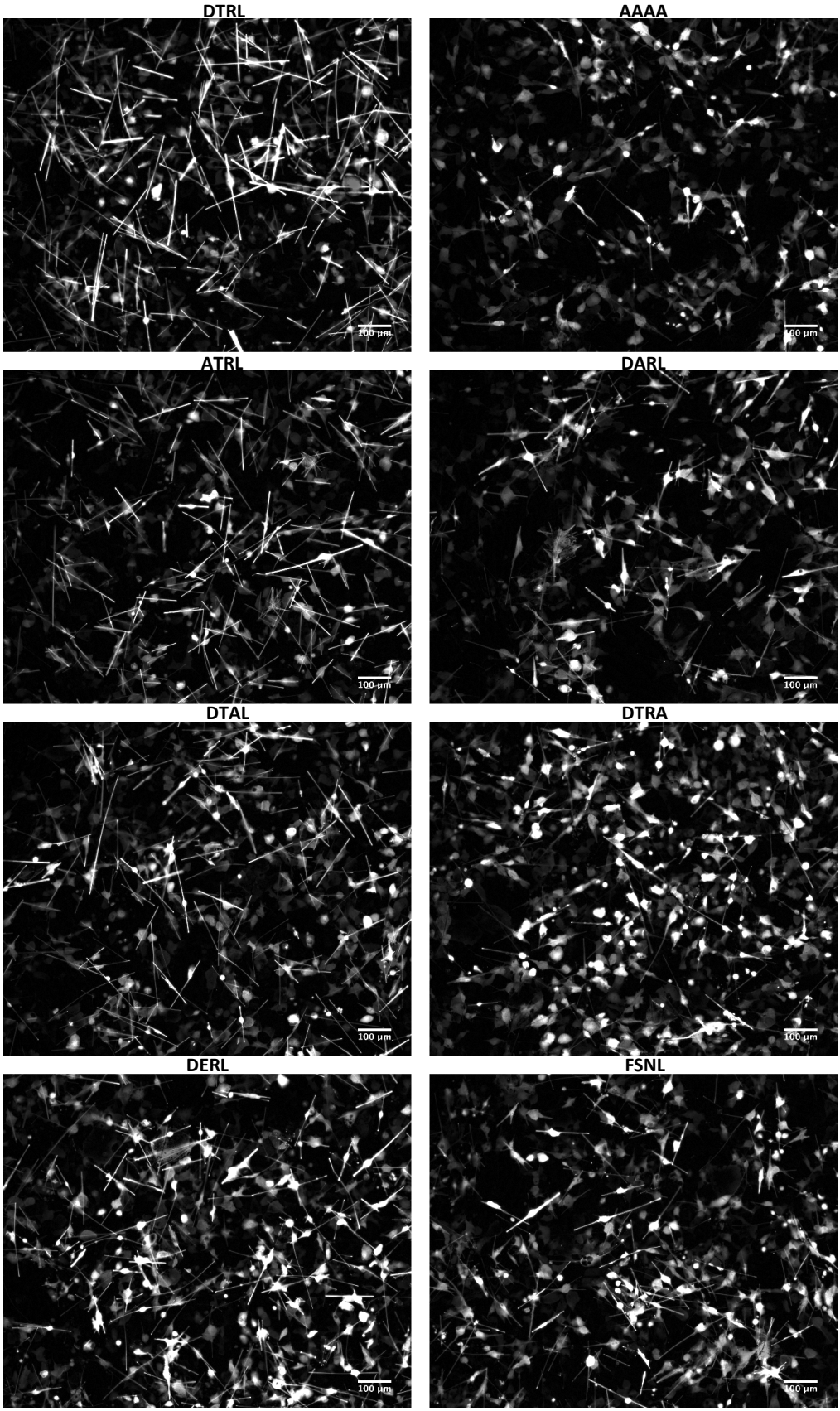
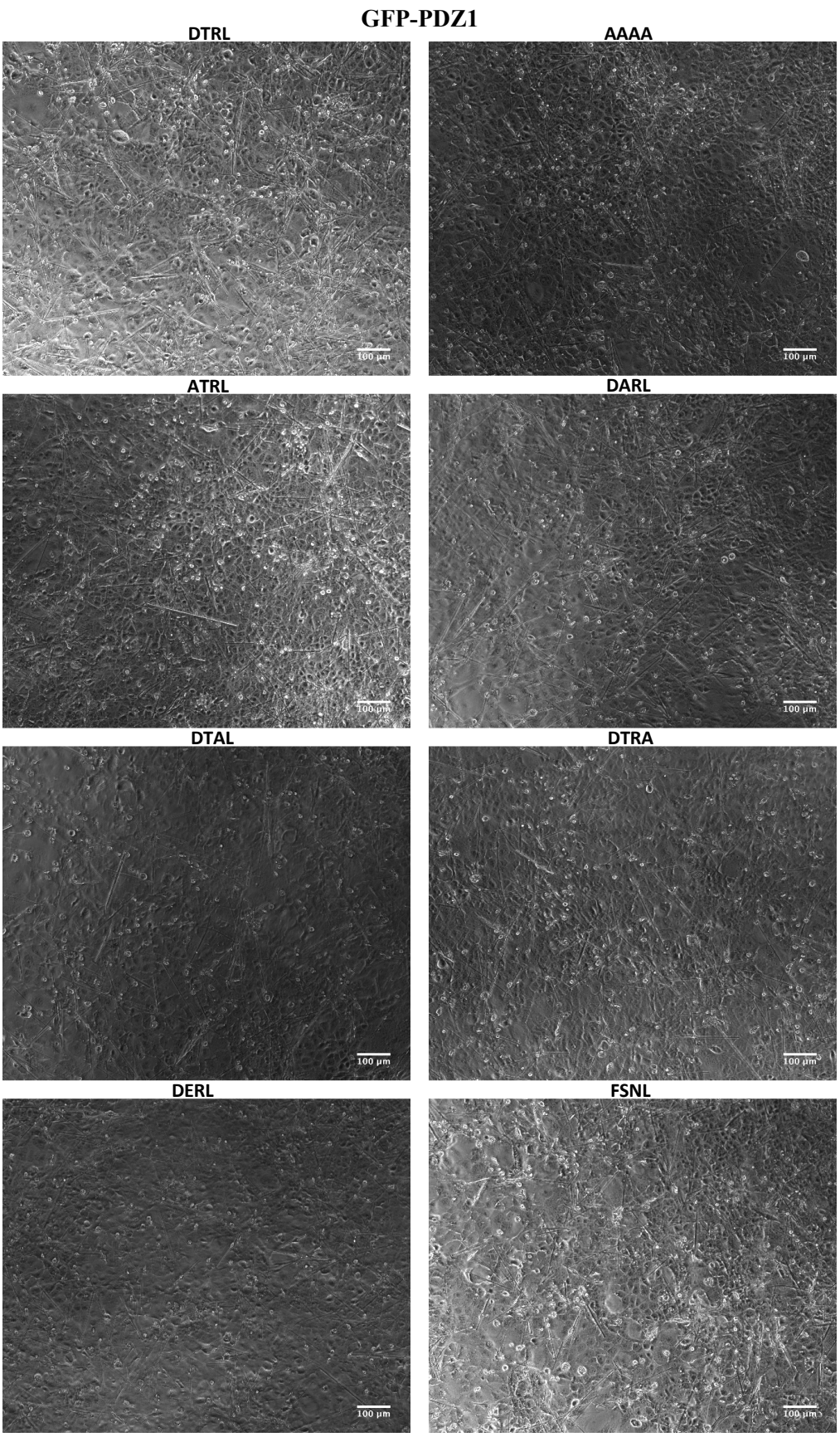


Figure S7. Representative GFP fluorescent images of COS-7 cells co-transfected with GFP-PDZ and iBox-PAKcat4-DTRL construct variants. Please note: Images for GFP-PDZ2 with iBox-

PAK4cat-DTRL and GFP-PDZ2 with iBox-PAK4cat-DTRA co-transfections have been reproduced from Fig. 5B.



GFP-PDZ2

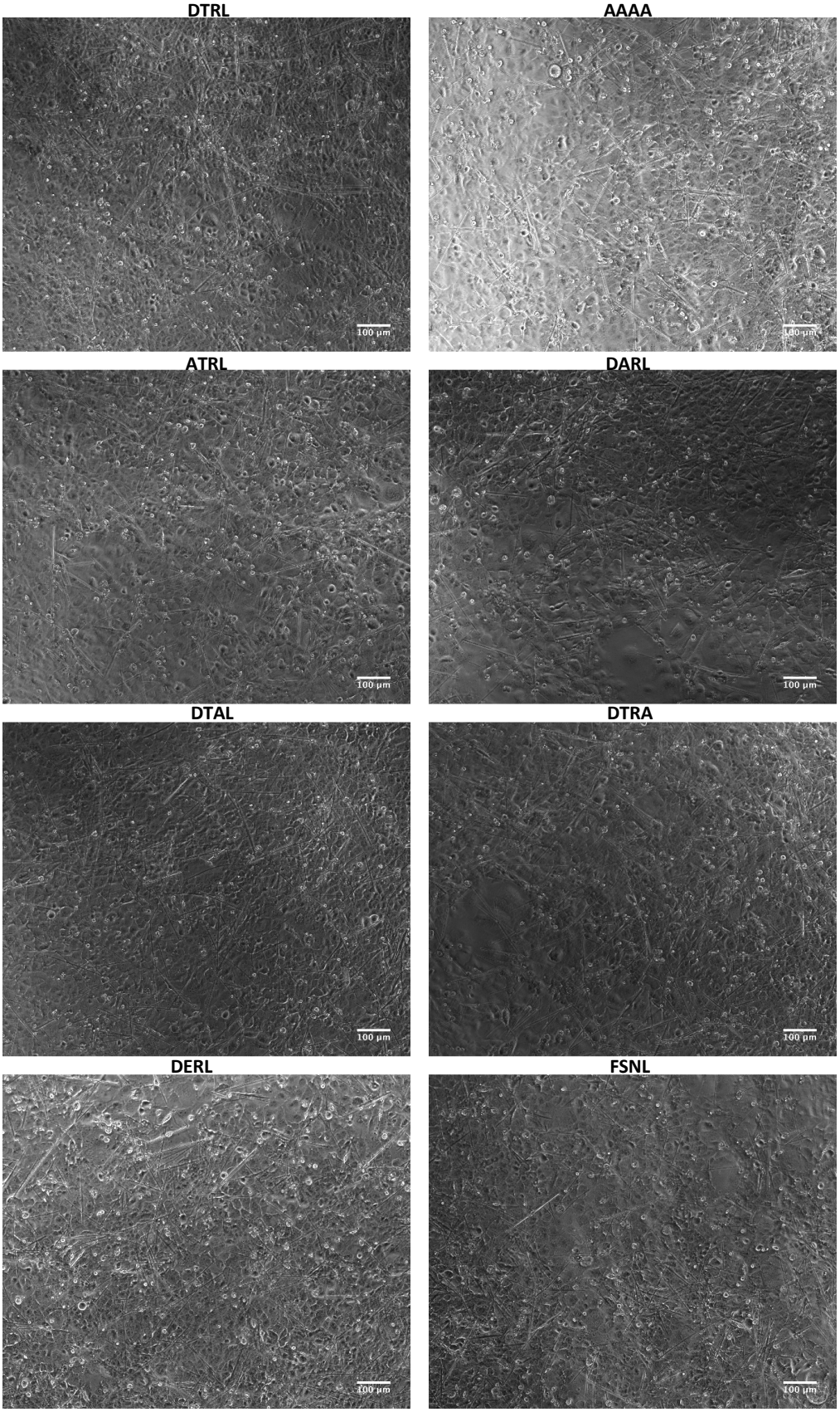


Figure S8. Corresponding light images for Figure S7.

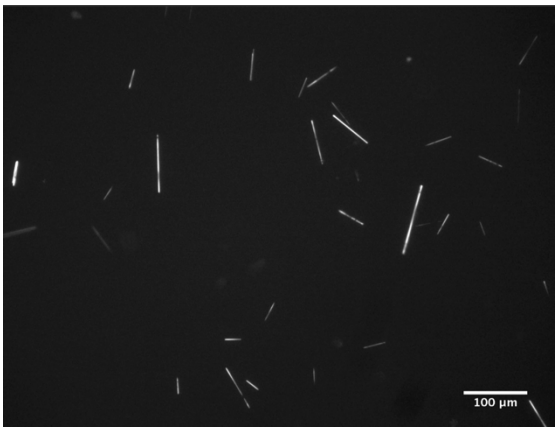
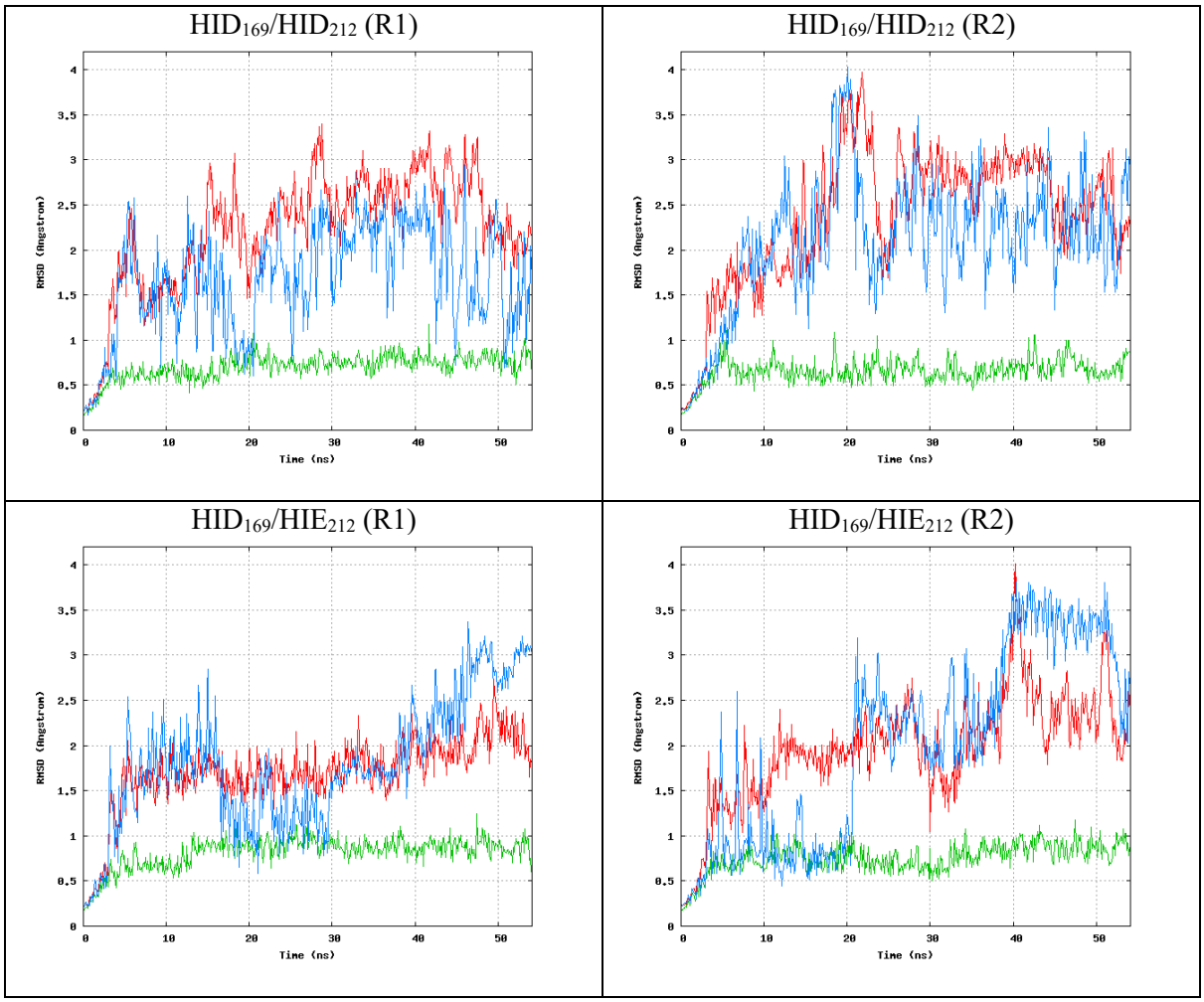


Figure S9. GFP fluorescent image of crystals obtained from lysed cells following co-transfection with GFP-PDZ1 and iBox-PAK4cat-DTRL.



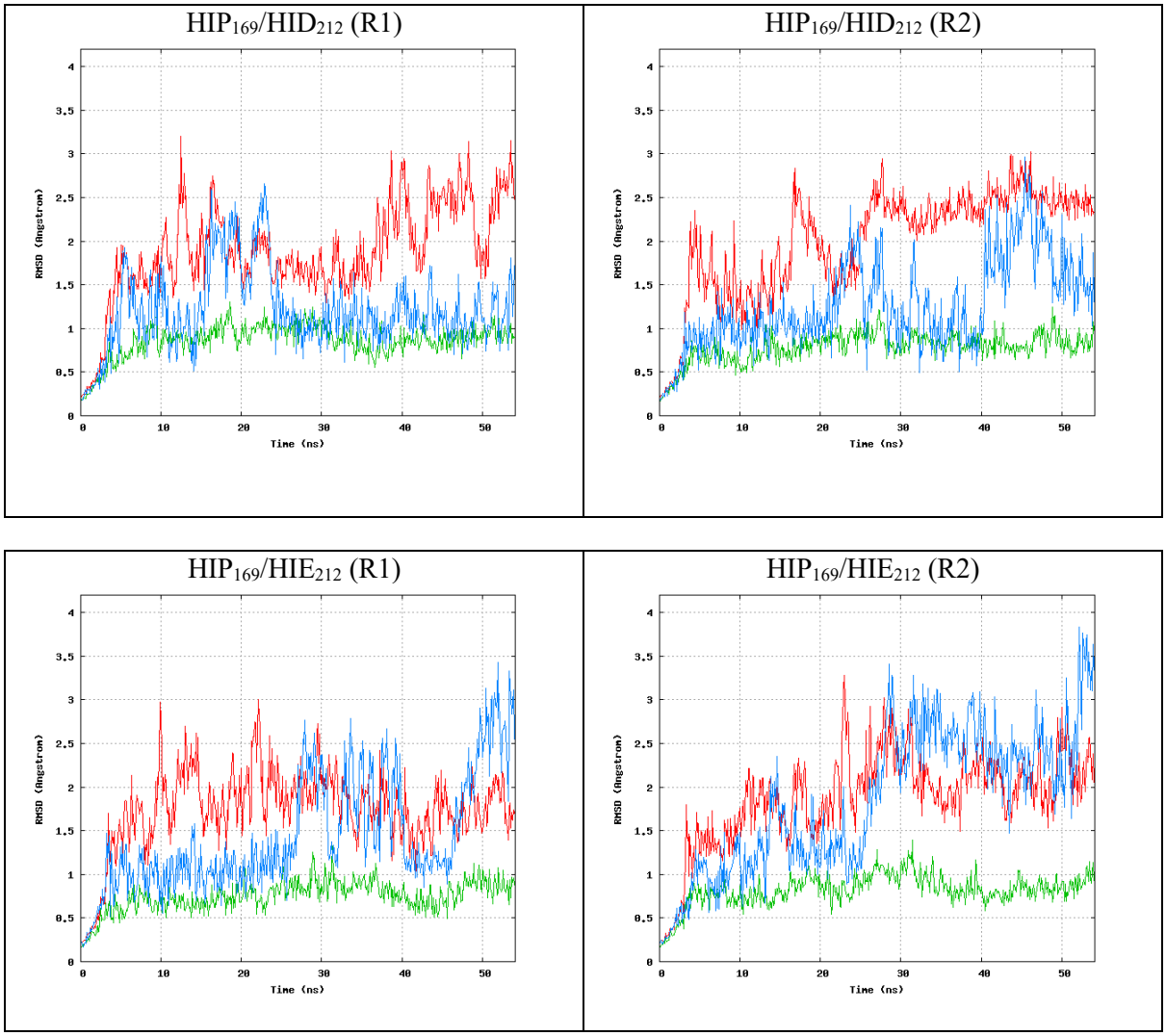
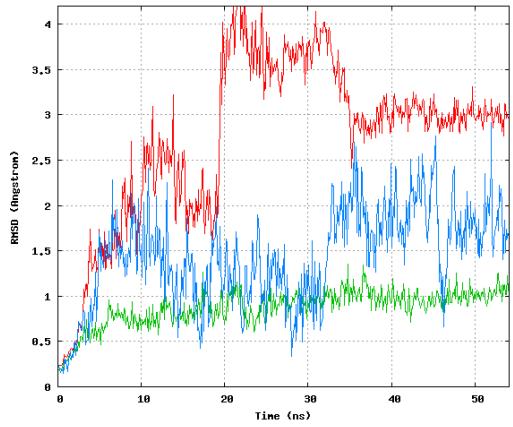
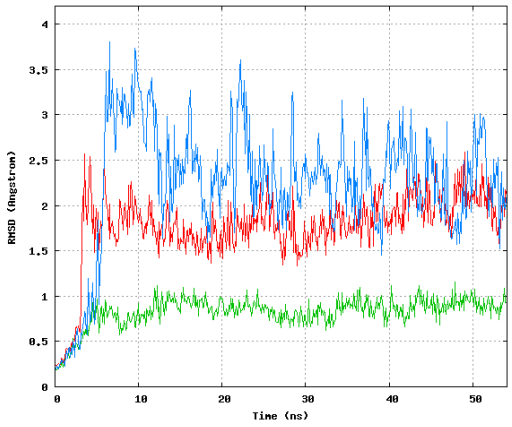


Figure S10. Root-mean-square-deviation (RMSD) of NHERF1 PDZ2 in complex with the CFTR PDZ-binding motif with different protonation states for H169 and H212. HIP (+1 charged, both δ- and ε-nitrogens protonated), HID (neutral, δ-nitrogen protonated), and HIE (neutral, ε-nitrogen protonated). R = replicate. RMSD for all Cα atoms is shown in red, the RMSD related to Cα atoms of the secondary structure elements of the complex is shown in green, and the RMSD of Cα atoms of the peptide is shown in blue.

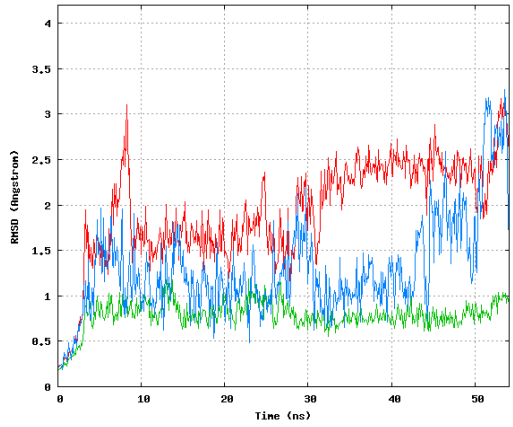
ATRL (R1)



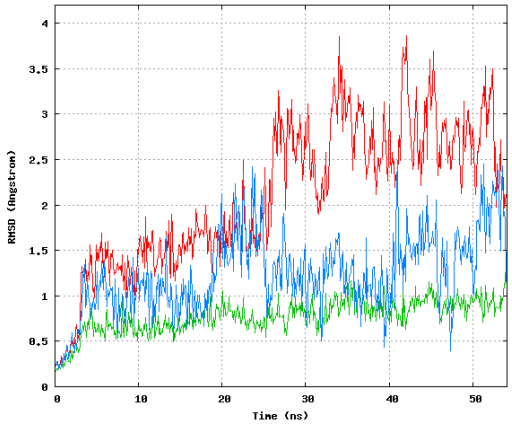
ATRL (R2)



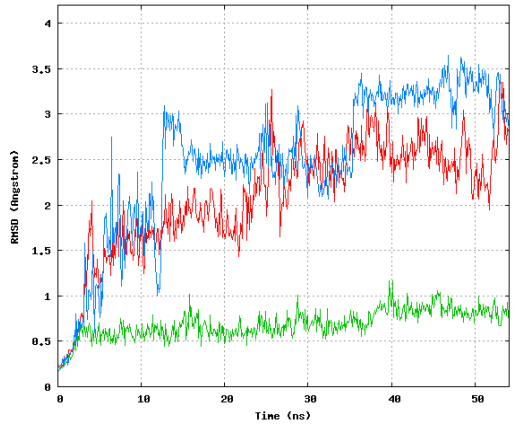
DARL (R1)



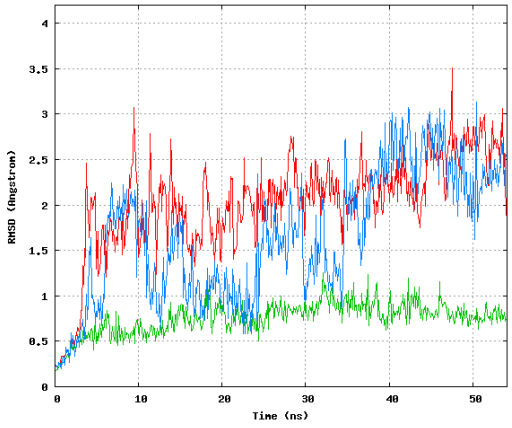
DARL (R2)



DTAL (R1)



DTAL (R2)



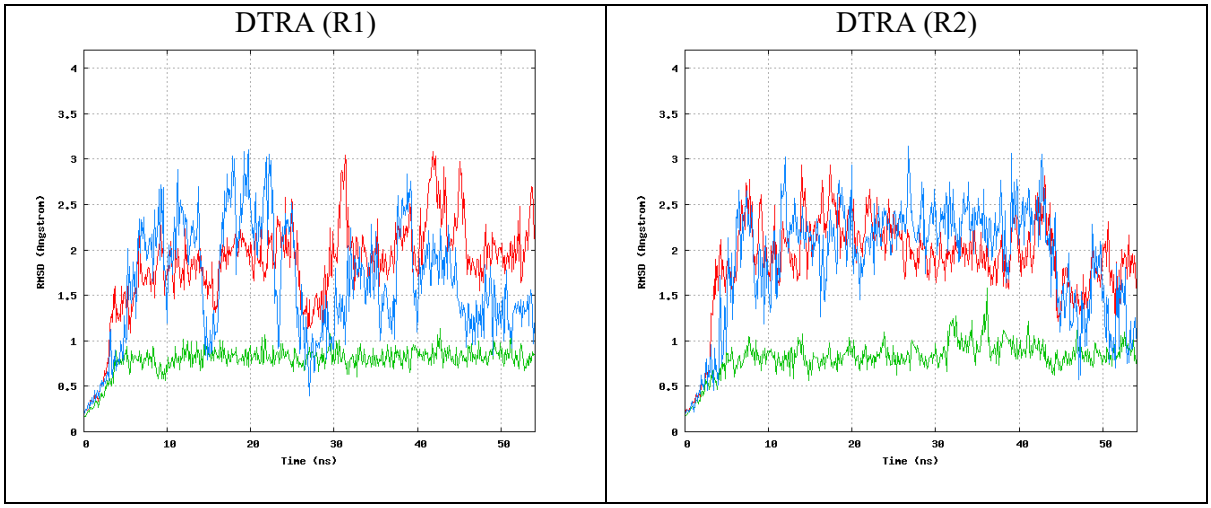


Figure S11. Root-mean-square-deviation (RMSD) of NHERF1 PDZ2 in complex with the CFTR PDZ-binding motif alanine substitution mutants. R = replicate. RMSD for all Cα atoms is shown in red, the RMSD related to Cα atoms of the secondary structure elements of the complex is shown in green, and the RMSD of Cα atoms of the peptide is shown in blue.

Space group	$P6_3$
Cell dimensions	
(a, b, c) (Å)	143.1, 143.1, 61.8
(α, β, γ) (°)	90.0, 90.0, 120.0
Resolution range (Å)	19.9-3.15 (3.21-3.15)
Unique reflections	13276 (633)
Completeness (%)	99.9 (99.4)
Multiplicity	3.6 (3.1)
R_{merge}	18.8 (57.6)
I/σ	6.3 (2.0)

Table S1. Diffraction statistics for the iBox-PDZ2-PAK4cat fusion crystal.

Movie S1. Fluorescent time-lapse comparison of COS-7 cells co-transfected with GFP-PDZ2 and iBox-PAK4cat constructs. iBox-PAK4cat-DTRL, left, or iBox-PAK4catDTRA, right.