SUPPLEMENTARY MATERIALS

Structural Elements That Govern Sec14-like Phosphatidylinositol Transfer Protein Sensitivities to Potent Small Molecule Inhibitors

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Legend to Supplementary Movie S1

Supplementary Movie S1. Molecular dynamics simulation of NPPM bound in the Sec14 hydrophobic cavity showing interconversion between two bound poses. The movie shows a 10 ns all atom MD simulation of a Sec14::NPPM 6748-481 complex in explicit waters. NPPM 6748-481 is rendered in ball & stick representation and water as anti-aliased stick model, protein secondary structure in ribbon cartoon and pocket residues rendered in stick models. Later in the movie, for clarity, only pocket residues are shown 4.5 Å around the NPPM 6748-481. Note the rotation of the activated aryl halide group of the NPPM to achieve alternate binding modes based on previous docking and mutagenesis studies (14). Numbering of residues is offset by -3, as numbering in homology model started from 1 while numbering started from 4 in the crystal structure template (PDB: 1AUA). So, residue Ser170 in homology model corresponds to residue Ser173 in the crystal structure.

Supplementary Figure Legends

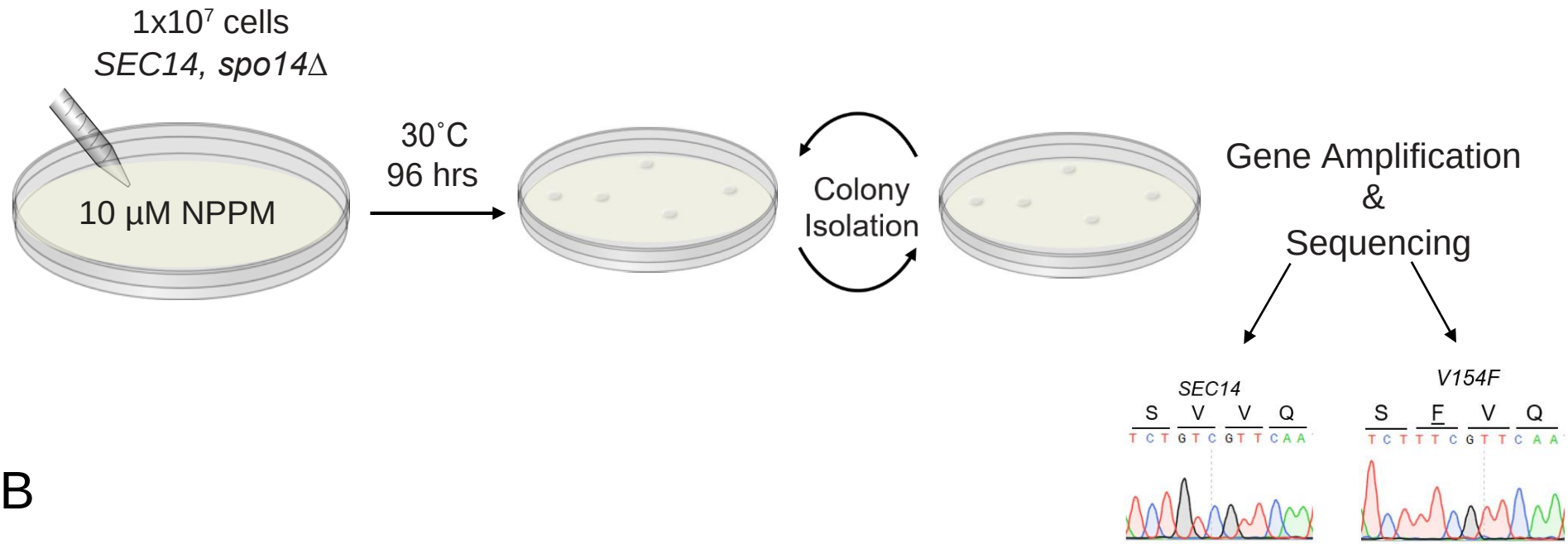
Supplementary Figure S1. Genetic screen for NPPM-resistant *SEC14* alleles. *A,* a schematic representation of the unbiased genetic screen for NPPM-resistant Sec14 proteins is illustrated. Approximately 10^7 cells were seeded onto each individual YPD agar plate supplemented with the appropriate NPPM (10μM final concentration). After the indicated period of incubation, NPPM resistant ($NPPM^R$) colonies were purified by two rounds of streaking for isolated colonies on NPPM-containing agar plates. The corresponding *SEC14* genes were amplified by PCR from genomic DNA prepared isolated $NPPM^R$ clones using the appropriate oligonucleotide primers (see Materials and Methods, Supplemental Table S1). Nucleotide sequences for each *SEC14* amplicon were determined and analyzed for missense substitutions. ***B,*** documents that the frequency of colony generation was inversely proportional to the inhibitor potency. Decreased potency of the challenge NPPM came with more rapidly arising $NPPM^R$ colonies. To reduce screen background (i.e. occurrence of $NPPM^R$ mutations that did not map to *SEC14*), a *pdr5*Δ strain that shows increased NPPM-sensitivity was used in a parallel genetic screen.

Supplementary Figure S2. Genetic screen for NPPM-resistant *SEC14* alleles. The indicated *SEC14* genes were integrated into the *MET17* locus of a *sec14-1^{ts} spo14Δ* strain and expressed under the control of the *S. cerevisiae SEC14* promoter to generate strains exhibiting 'physiological' levels of each Sec14 variant. The integrants were subsequently dilution spotted onto YPD plates supplemented with vehicle control DMSO or 20μM NPPM, as indicated at top, and incubated at 48hrs at the indicated temperatures. The mock condition documents the phenotype of an isogenic strain where a *SEC14*-less integration cassette was transplaced into the *MET17* locus. That expression of each Sec14 protein was sufficient to rescue *sec14-1^{ts}* growth defects at the restrictive temperature of 37°C is demonstrated by comparison of

the growth profiles in the left (25°C) and center (37°C) panels of the integrants relative to mock controls. The NPPM^{R} phenotypes are displayed in the right panel. The plates were incubated for 48 h at the indicated temperatures before imaging. The NPPM481-resistance phenotypes were scored at 25°C. Scale bar, 1cm.

Supplementary Figure S1

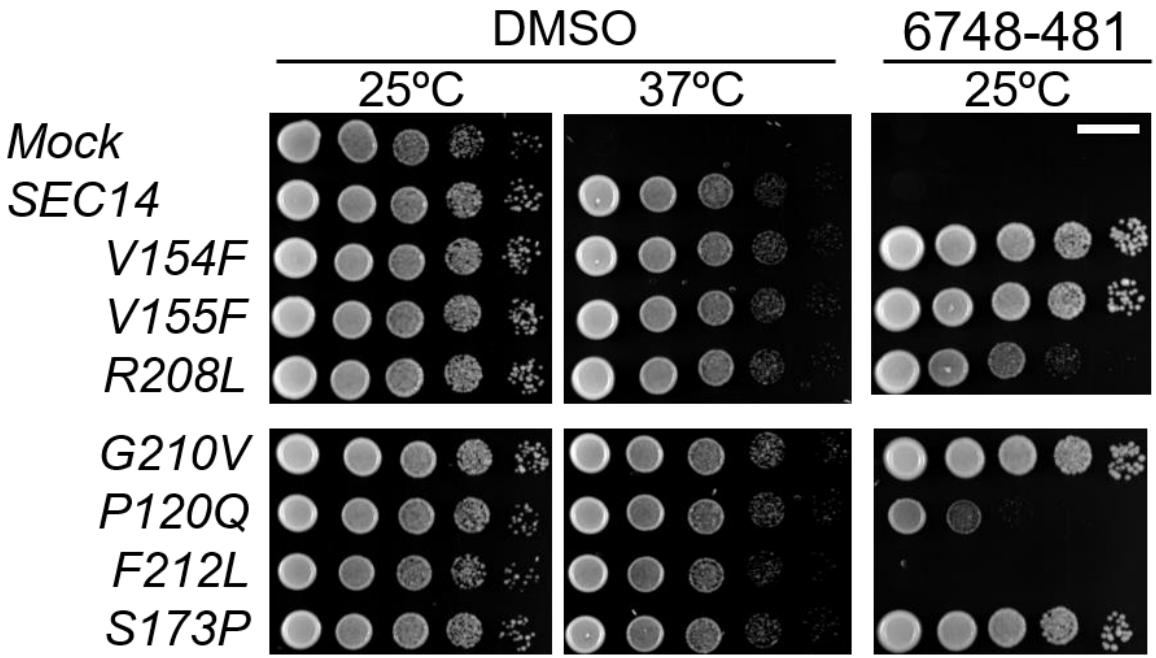
A



B

NPPM		Appearance (hrs.)	Frequency (cell/generation)	Sequenced
4130-1276		48	3×10^{-5}	X
67107-49		48	3×10^{-6}	X
6748-481	***PDR5***	96	1×10^{-7}	40
	***pdr5*Δ**	96	3×10^{-8}	5

Supplementary Figure S2



Supplementary Table S1.

A. Gene replacement cassette plasmids

Plasmid	Description	Origin
pVB9	pBSK(*pdr5Δ::GFP, KanMX*)	This study
pVB16(mock)	pBSK(*leu2Δ::HIS3*)	This study
pVB18	pBSK(*leu2Δ::SEC14,HIS3*)	This study
pVB70	pVB18(*leu2Δ::SEC14 S173C,HIS3*)	This study
pKM16	pVB18(*leu2Δ::SEC14 P120Q,HIS3*)	This study
pDK154	pVB18(*leu2Δ::SEC14 V154F,HIS3*)	This study
pDK155	pVB18(*leu2Δ::SEC14 V155F,HIS3*)	This study
pKM14	pVB18(*leu2Δ::SEC14 S173P,HIS3)*	This study
pDK156	pVB18(*leu2Δ::SEC14 R208L,HIS3*)	This study
pDK157	pVB18(*leu2Δ::SEC14 G210V,HIS3*)	This study
pKM17	pVB18(*leu2Δ::SEC14 F212L,HIS3*)	This study
pDK257	pVB18(*leu2Δ::SEC14 V154M, V155C ,HIS3*)	This study
pDK9	pVB18(*leu2Δ::SEC14*$_{CA}$*, HIS3*)	This study
pDK265	pVB18(*leu2Δ::SEC14*$_{CA}$ *M154V, C155V, HIS3*)	This study
pDK248	pVB18(*leu2Δ::SEC14*$_{KL}$*, HIS3*)	This study
pDK266	pVB18(*leu2Δ::SEC14*$_{KL}$ *F152V, HIS3*)	This study
pDK247	pVB18(*leu2Δ::SEC14*$_{CG}$*, HIS3*)	This study
pDK267	pVB18(*leu2Δ::SEC14*$_{CG}$ *V152F, HIS3*)	This study
pDK268	pVB18(*leu2Δ::SEC14*$_{CG}$*V152F, V153F, HIS3*)	This study
pDK80	pBSK(*met17Δ::URA3*)	This study
pDK81	pBSK(*met17Δ::SEC14,URA3)*	This study
pDK239	pDK81(*met17Δ::SEC14 V154F,URA3*)	This study
pDK240	pDK81(*met17Δ::SEC14 V154A,URA3*)	This study
pDK241	pDK81(*met17Δ::SEC14 V154E,URA3*)	This study
pDK242	pDK81(*met17Δ::SEC14 V154Y,URA3*)	This study
pDK243	pDK81(*met17Δ::SEC14 V155F,URA3*)	This study
pDK244	pDK81(*met17Δ::SEC14 V155A,URA3*)	This study
pDK245	pDK81(*met17Δ::SEC14 V155E,URA3*)	This study
pDK246	pDK81(*met17Δ::SEC14 V155Y,URA3*)	This study
pDK280	pDK81(*met17Δ::SEC14 F212L,URA3*)	This study
pDK281	pDK81(*met17Δ::SEC14 S173P,URA3*)	This study
pDK282	pDK81(*met17Δ::SEC14 P120Q,URA3*)	This study
pDK283	pDK81(*met17Δ::SEC14 R208L,URA3*)	This study

B. Yeast plasmids.

Plasmid	Description	Origin
pCTY1716	YCp(*SFH1, URA3*)	(22)
pCTY1727	YCp(*SFH1 E126A, URA3*)	(22)
pDK252	YCp(*SFH1 F156V, URA3*)	This study
pDK255	YCp(*SFH1 F156V A157V, URA3*)	This study

Supplementary Table S2. Primers used.

DKO98	To subclone *SEC14* into pVB16 at *SacII*, *SphI* sites	aaaccgcggatggttacagtatgttgttgc
DKO99		tttgcatgctcatttcatcgaaaaggcttccgg
DKO107	To generate *SEC14* V154F by mutagenesis	cttggtttgggaatacgaatctttcgttcaatacagattacctgcc
DKO108		ggcaggtaatctgtattgaacgaaagattcgtattcccaaaccaag
DKO105	To generate *SEC14* V155F by mutagenesis	ggtttgggaatacgaatctgtctttcaatacagattacctgcctg
DKO106		caggcaggtaatctgtattgaaagacagattcgtattcccaaacc
DKO201	To generate *SEC14* S173P by mutagenesis	ctggtcacctagtg gaaactccatgtacaattat ggatttgaaa g
DKO202		ctttcaaatccataattgtacatggagtttccactaggtgaccag
DKO109	To generate *SEC14* R208L by mutagenesis	cataagtcaaaactattaccccgaacttatgggtaaattttacatcatc
DKO110		gatgatgtaaaatttacccataagttcggggtaatagttttgacttatg
DKO111	To generate *SEC14* G210V by mutagenesis	caaaactattaccccgaacgtatggttaaattttacatcatcaacgcgcc
DKO112		ggcgcgttgatgatgtaaaatttaaccatacgttcggggtaatagttttg
KMO104	To generate *SEC14* P120Q by mutagenesis	cataaaaccgataaagatggccgc caa gtatattttgaagaattaggtg
KMO105		cacctaattcttcaaaatatacttggcggccatctttatcggttttatg
KMO106	To generate *SEC14* F212L by mutagenesis	ctattaccccgaacgtatgggtaaa ctt ttttacatcatcaacgcgccattc
KMO107		gaatggcgcgttgatgatgtaaaaaagtttacccatacgttcggggtaatag
DKO189	To generate *SEC14* V155A by mutagenesis	ggtttgggaatacgaatctgtcgctcaatacagattacctgcctgttc
DKO190		gaacaggcaggtaatctgtattgagcgacagattcgtattcccaaacc
DKO191	To generate *SEC14* V155E by mutagenesis	ggtttgggaatacgaatct gtcgaacaata cagattacct gcctgttc
DKO192		gaacaggcaggtaatctgtattgttcgacagattcgtattcccaaacc
DKO193	To generate *SEC14* V155Y by mutagenesis	ggtttgggaatacgaatctgtctatcaata cagattacct gcctgttc
DKO194		gaacaggcaggtaatctgtattgatagacagattcgtattcccaaacc
DKO195	To generate *SEC14* V154A by mutagenesis	cttggtttgggaatacgaatctgccgttcaata cagattacct gcctg
DKO196		caggcaggtaatctgtattgaacggcagattcgtattcccaaaccaag
DKO197	To generate *SEC14* V154E by mutagenesis	cttggtttgggaatacgaatctgaagttcaata cagattacct gcctg
DKO198		caggcaggtaatctgtattgaacttcagattcgtattcccaaaccaag
DKO199	To generate *SEC14* V154Y by mutagenesis	cttggtttgggaatacgaatcttacgttcaata cagattacct gcctg
DKO200		caggcaggtaatctgtattgaacgtaagattcgtattcccaaaccaag
DKO335	To generate *SEC14* V154M by mutagenesis	gaaaaacttggtttgggaatacgaatctatggttcaatacagattacctgc
DKO336		gcaggtaatctgtattgaaccatagattcgtattcccaaaccaagtttttc
DKO337	To generate *SEC14* V154M, V155C by mutagenesis	gaaaaacttggtttgggaatacgaatct atg tgt caatacagattacctgcctgttc
DKO338		gaacaggcaggtaatctgtattgacacatagattcgtattcccaaaccaagttttc
DKO1	To amplify $SEC14_{CA}$ from gDNA and sub-clone into pVB16 at *SacII*, *SphI* sites	aaaccgcggatgactacgatgactactgaagaaatattggc
DKO2		tttgcatgcttaaatgttataagctctaggacattcacc
DKO14	To sub-clone 8His-$SEC14_{CA}$ into pET28b at *NcoI*, *SacI* sites	aaccatgggtcatcatcatcatcatcatcatatgactacgatgactactgaa
DKO15		aagagctctta aatgttataagctctaggacattcacc
CA84	To generate $SEC14_{CA}$ M154V, C155V by mutagenesis	cttggtatgggaatatgaagccgtggttcaatatcgtttacctgcatgttc
CA85		gaacatgcaggtaaacgatattgaaccacggcttcatattcccataccaag
KL100	To amplify $SEC14_{KL}$ from gDNA and sub-clone into pVB16 at *SacII*, *SphI* sites	aaccgcggatggtaagtgaacaagaaattttag
KL101		ttgcatgcttacaattgaaaagcctttggagcttc
KL90	To sub-clone 8His-$SEC14_{KL}$ into pET28b at *NcoI*, *SacI* sites	aaccatgggtcatcatcatcatcatcatcatatggtaagtg aacaagaaattttag
KL91		aagagctcttacaattgaaaagcctttggagcttcaccttcaggtccaatgtat
KL92	To generate $SEC14_{KL}$ without internal *NcoI* site by silent base-pair exchange and mutagenesis	ctatatctatccgatattggtccctggagagaggaggaatacattggacc
KL93		ggtccaatgtattcctcctctctccagggaccaatatcggatagatatag

KL95	To generate *SEC14*$_{KL}$ F152V by mutagenesis	gaacttggtctgggaatacgaagctgttgttagatacagattgcctgcatgt tc
KL96		gaacatgcaggcaatctgtatctaacaacagcttcgtattcccagaccaagttc
CG102	To amplify *SEC14*$_{CG}$ from gDNA and sub-clone into pVB16 at *SacII*, *SphI* sites	aaccgcggatggttagtgaagcggagtttttg
CG103		ttgcatgcttatttcatggaaaacatctc
CG88	To sub-clone 8His-*SEC14*$_{CG}$ into pET28b at *NcoI*, *SacI* sites	aaccatgggtcatcatcatcatcatcatcatatg gttagtgaagcggagttttggc
CG89		ttgagctcttatttcatggaaaacatctctggggcttcaccttc
CG104	To generate *SEC14*$_{CG}$ without internal *NcoI* site by silent base-pair exchange and mutagenesis	cctgtacttgtccgacatcggcccctggagagacgccaagtacatc
CG105		gatgtacttggcgtctctccaggggccgatgtcggacaagtacagg
CG112	To generate *SEC14*$_{CG}$ F152V by mutagenesis	gaacttggtgtgggagtacgagtcc ttt gtcaactacagactgcctgcttgctc
CG113		gagcaagcaggcagtctgtagttgacaaaggactcgtactcccacaccaagttc
CG114	To generate *SEC14*$_{CG}$ F152V, F153V by mutagenesis	gaacttggtgtgggagtacgagtccttttttcaactacagactgcctgcttgctc
CG115		gagcaagcaggcagtctgtagttcaaaaaggactcgtactcccacaccaagttc
DKO94	To sub-clone *SFH1* into pVB16 at *SacII*, *SphI* sites	aaaccgcggatgacaaccagcatactc
DKO95		tttgcatgcttagctggtaacagtaaatttac
DKO163	To generate *SFH1* F156V by mutagenesis	gaaacttagtcaaggagtacgaattagttgccacgtaccgggtcccagcgtgttcg
DKO164		cgaacacgctgggacccggtacgtggcaactaattcgtactccttgactaagtttc
DKO147	To generate *SFH1* F156V, A157V by mutagenesis	cttagtcaaggagtacgaattagttgtcacgtaccgg gtcccagcgt gttcg
DKO148		cgaacacgctgggacccggtacgtgacaactaattcgtactccttgactaag

Supplementary Table S3. Protein expression plasmids.

Plasmid	Description	Origin
pRE1201	pET28b (His$_8$-*SEC14*)	(9)
pDK221	pET28b (His$_8$-*SEC14 P120Q*)	This study
pDK150	pET28b (His$_8$-*SEC14 V154F*)	This study
pDK152	pET28b (His$_8$-*SEC14 V155F*)	This study
pRE1270	pET28b (His$_8$-*SEC14 S173C*)	(14)
pDK171	pET28b (His$_8$-*SEC14 S173P*)	This study
pDK151	pET28b (His$_8$-*SEC14 R208L*)	This study
pDK153	pET28b (His$_8$-*SEC14 G210V*)	This study
pDK222	pET28b (His$_8$-*SEC14 F212L*)	This study
pDK31	pET28b (His$_8$-*SEC14$_{CA}$*)	This study
pDK212	pET28b (His$_8$-*SEC14$_{CA}$ M154V,C155V*)	This study
pDK262	pET28b (His$_8$-*SEC14$_{KL}$*)	This study
pDK269	pET28b (His$_8$-*SEC14$_{KL}$ F152V*)	This study
pDK261	pET28b (His$_8$-*SEC14$_{CG}$*)	This study
pDK270	pET28b (His$_8$-*SEC14$_{CG}$ V152F*)	This study
pDK271	pET28b (His$_8$-*SEC14$_{CG}$ V152F, V153F*)	This study
pRE1227	pET28b (His$_8$-*SFH1*)	(9)
pRE1234	pET28b (His$_8$-*SFH1 E126A*)	(22)
pDK77	pET28b (His$_8$-*SFH1 F153V, L176M, I193V, V196A, A197S, Q204A, V227F*)	This study
pDK149	pET28b (His$_8$-*SFH1 E126A,F156V*)	This study
pDK126	pET28b (His$_8$-*SFH1 E126A,F156V,A157V*)	This study

Supplementary Table S4. Intrinsic Sfh1^{Q204,6X} PtdIns-transfer activity.

Protein	Activity (Transfer as % of input [^{3}H]-PtdIns)	Total Input [^{3}H]-PtdIns (c.p.m.)	Background (c.p.m.)
Sec14	18-26	8915-10863	645-955
Sfh1 ^{Q204,6X}	22-25	8915-10863	645-955

Supplementary Table S5. Intrinsic PtdIns-transfer activities for NPPM^{R} Sec14 proteins.

Protein	Activity (Transfer as % of input [^{3}H]-PtdIns)	Total Input [^{3}H]-PtdIns (c.p.m.)	Background (c.p.m.)
Sec14	16-28	7410-10100	530-570
P120Q*	-	-	-
V154F	13-18	8510-12125	780-1630
V155F	10-20	7400-10915	570-870
S173P*	-	-	-
R208L	6-10	8500-12125	570-1630
G210V*	-	-	-
F212L	13-21	7805-10400	590-870

*Insufficient activity for confident assessment (<3% transfer).

Supplementary Table S6. Intrinsic PtdIns-transfer activities of Sec14-like PITPs and VV-motif variants.

Protein	Basal Activity (%)	Total Input (c.p.m.)	Background (c.p.m.)
Sec14	18-26	8915-10863	645-955
$Sec14_{CA}$	24-27	10243-11080	810-1054
$Sec14_{CA}^{M154V, C155V}$	12-18	8955-10470	960-1105
$Sec14_{CG}$	22-24	10787-11113	1547-1669
$Sec14_{KL}$	20-23	10585-11240	1460-1517
$Sec14_{KL}^{F152V}$	25-27	9421-11133	1226-1330
$Sfh1^{E126A}$	18-26	8716-8976	645-955
$Sfh1^{E126A, F156V}$	19-24	9220-10878	825-980
$Sfh1^{E126A, F156V, A157V}$	13-16	10510-11285	834-1060