



Protein Purification
EEA1 truncate (GST-)
USF-2 SWAP
His-6/13 truncate

in vitro Binding Assay

14.11.04 ~
Oatashi



Endosome Fusion Assay ①
010604 ~

Oatashi



FALKEN

plasmid construction

SWARF

Oatashi



FISH Literature

SWARF proteins
5x12 L V11-V104
5x17



Soy bean Liposome

BHK-endo Liposome ①

recruitment Assay

PRA-1 purification

010605 ~

060906
Oatashi



LEITZ

Endosome Fusion Recruitment Assay ②

phosphorylation of EEA1
USF-1 USUP

080904 ~
270505

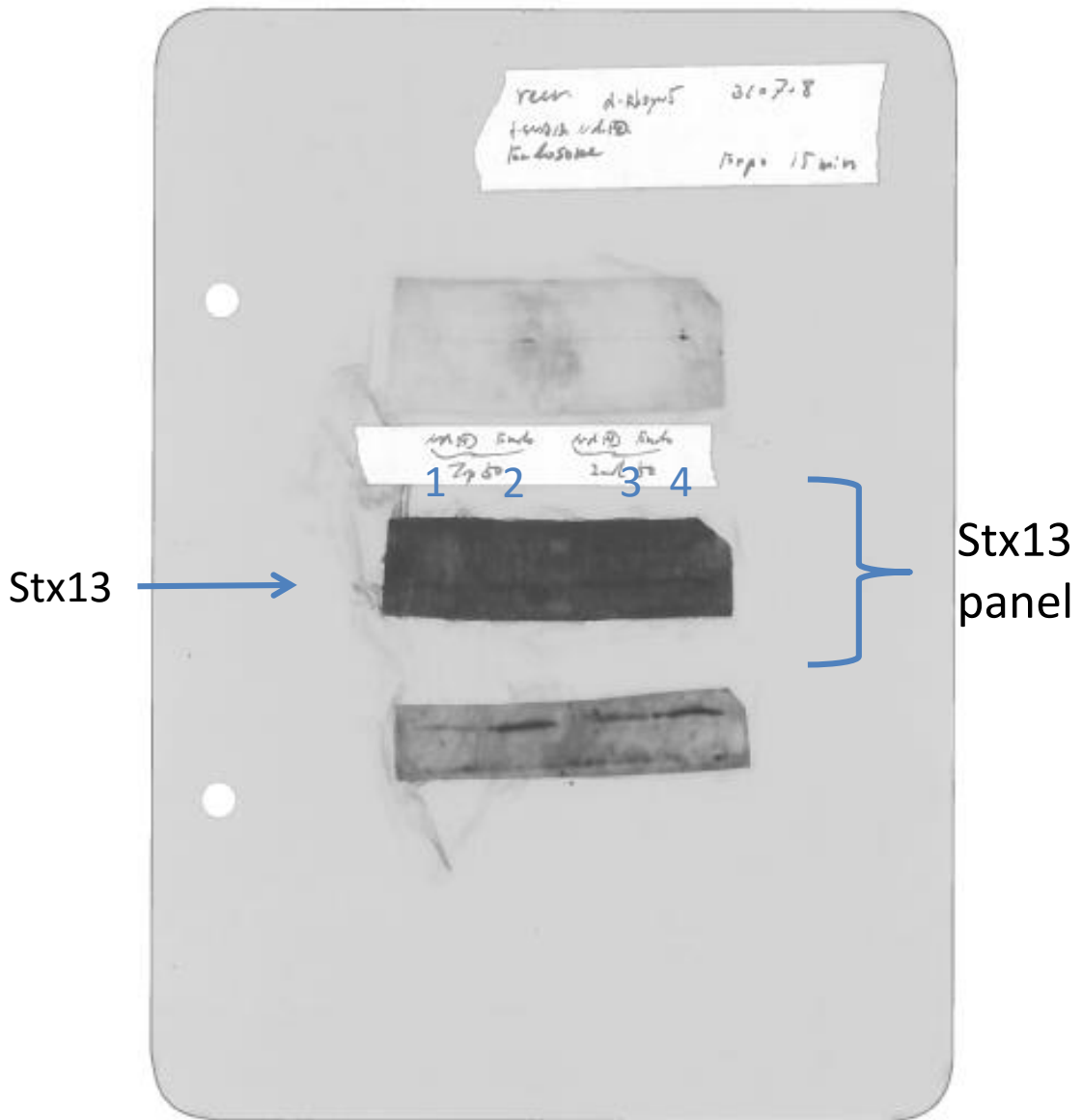


Protein purification

Baculo E. Coli HEK 293

Oatashi





Scan-1) Done at 31th Luly 2008

Second panel shows syntaxin13 blots.

- lane 1 : proteoliposomes with Stx13-Stx6-Vti1a-Pra1
- lane 2 : HeLa endosome
- lane 3 : proteoliposomes with Stx13-Stx6-Vti1a-Pra1
- lane 4 : HeLa endosom



Scan-4) Done at 31th July 2008

Second panel shows syntaxin13 blots.

lane 1 : proteoliposomes with Stx13-Stx6-Vti1a-Pra1

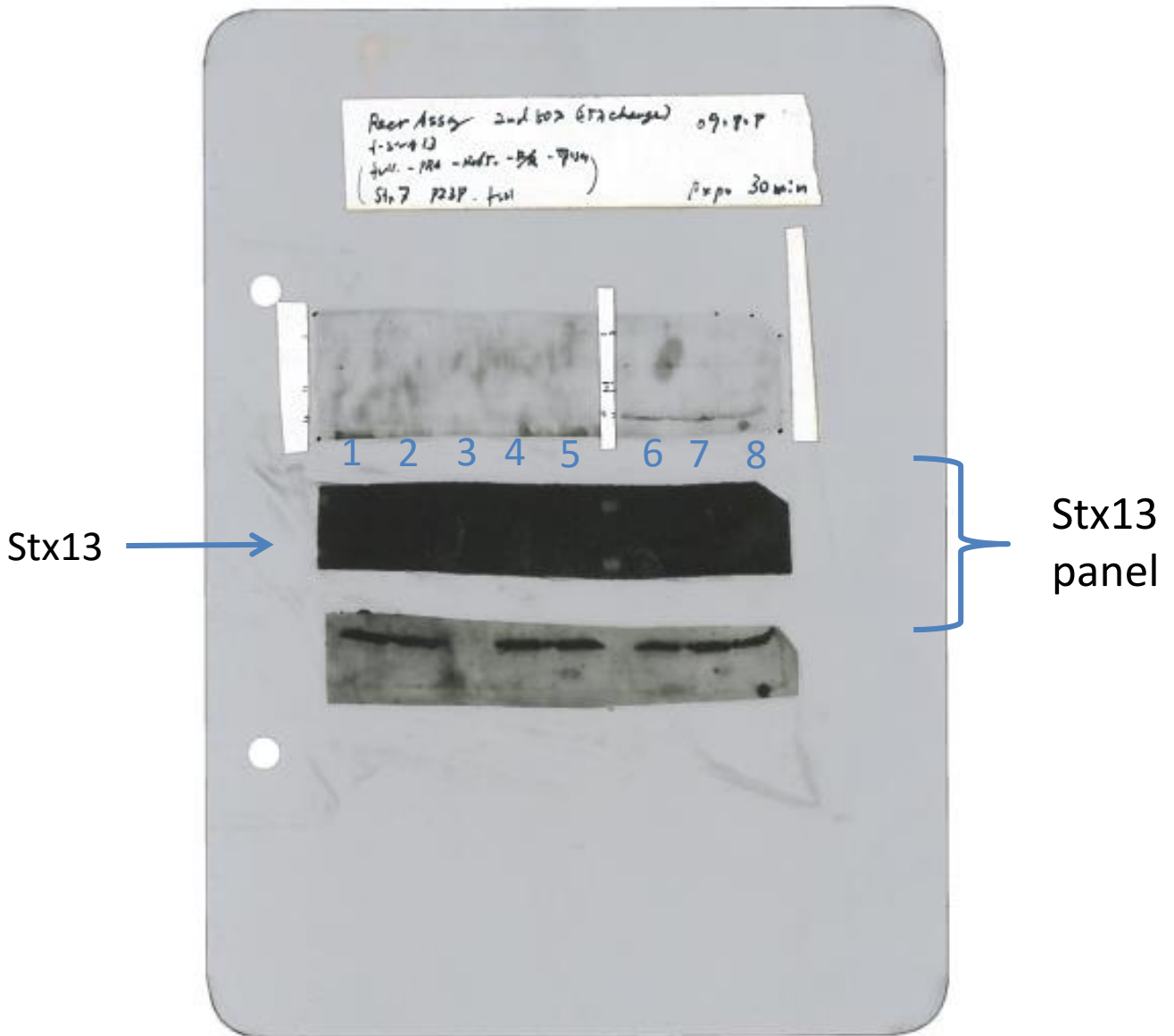
lane 2 : HeLa endosome

lane 3 : proteoliposomes with Stx13-Stx6-Vti1a-Pra1

lane 4 : HeLa endosome

The bands Fig. 2 lane 4, 5 correspond to the band on lane 3

The band Fig. 2 lane 6 corresponds to the band on lane 4



Scan-2) Done at 9th August 2008

Second panel shows syntaxin13 blots.

lane 1: proteoliposomes with Stx13-Stx6-Vti1a-Pra1 (full-set)

lane 2: proteoliposomes with Stx13-Stx6-Vti1a without Pra1

lane 3: proteoliposomes with Stx13-Stx6-Vti1a-Pra1 (w/o Rab5)

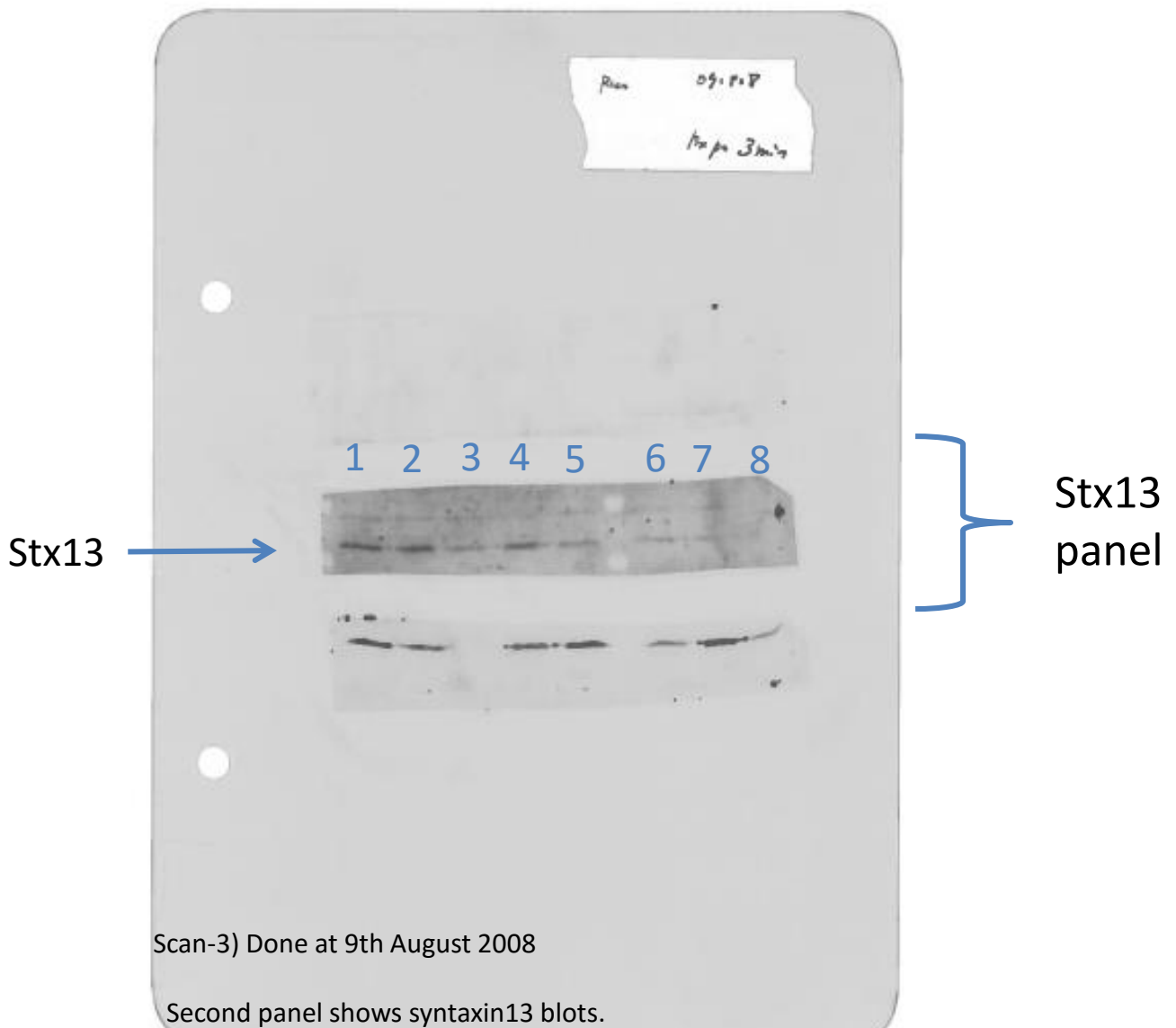
lane 4: proteoliposomes with Stx13-Stx6-Vti1a-Pra1 (w/o Rabaptin5/Rabex5)

lane 5: proteoliposomes with Stx13-Stx6-Vti1a-Pra1 (w/o Vpa34/p150)

lane 6: Stx7 proteoliposomes with Stx6-Vti1a-Pra1

lane 7: PI3P proteoliposomes with Stx13-Stx6-Vti1a-Pra1

lane 8: proteoliposomes with Stx13-Stx6-Vti1a-Pra1



Second panel shows syntaxin13 blots.

lane 1: proteoliposomes with Stx13-Stx6-Vti1a-Pra1 (full-set)

lane 2: proteoliposomes with Stx13-Stx6-Vti1a without Pra1

lane 3: proteoliposomes with Stx13-Stx6-Vti1a-Pra1 (w/o Rab5)

lane 4: proteoliposomes with Stx13-Stx6-Vti1a-Pra1 (w/o Rabaptin5/Rabex5)

lane 5: proteoliposomes with Stx13-Stx6-Vti1a-Pra1 (w/o Vpa34/p150)

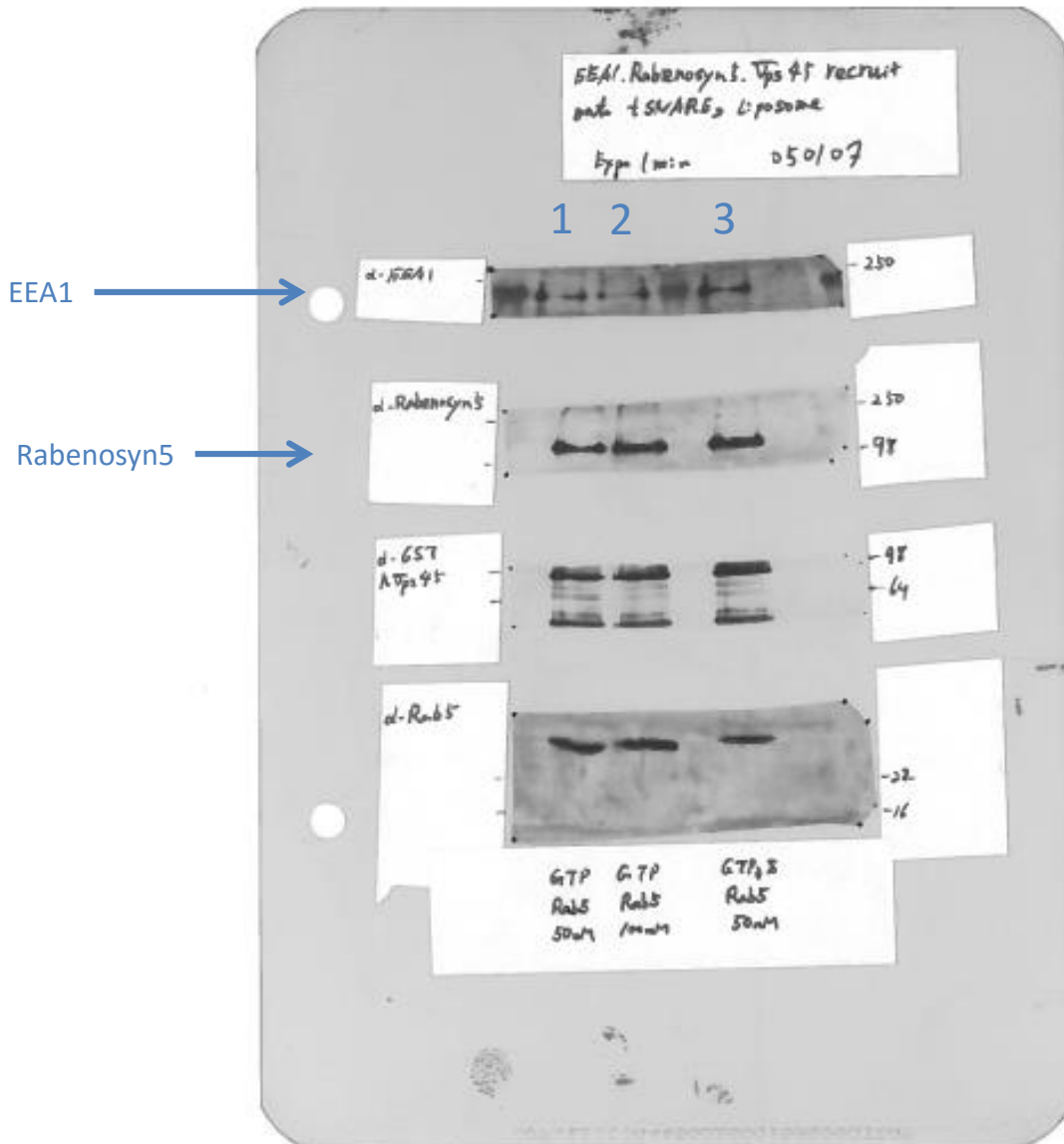
lane 6: Stx7 proteoliposomes with Stx6-Vti1a-Pra1

lane 7: PI3P proteoliposomes with Stx13-Stx6-Vti1a-Pra1

lane 8: proteoliposomes with Stx13-Stx6-Vti1a-Pra1

The bands Fig. 2 lane 1, 3 correspond to the band on lane 5

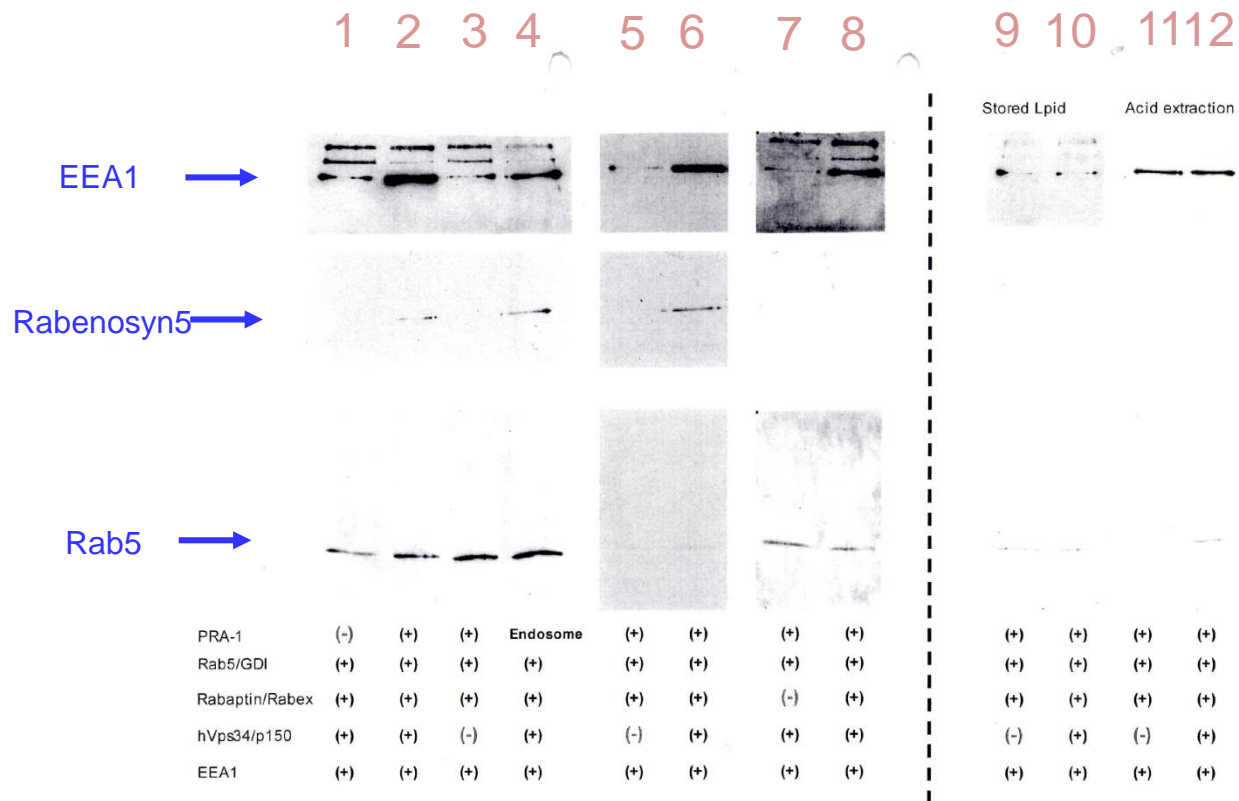
The bands Fig. 2 lane 2 correspond to the band on lane 4



Scan-5) Done at 5th January 2007

Proteoliposomes contain Stx13, Stx6, Vti1 and Pra1.
They were incubated with Rab5/GDI, Rabaptin5/Rabex5, hVps34/p150, EEA1 and Rabenosyn5/hVps45.

lane 1: 50nM of Rab5/Rab-GDI
lane 2: 100nM of Rab5/Rab-GDI
lane 3: 50nM of Rab5/Rab-GDI and GTP γ S



Proteoliposomes contain Stx13, Stx6, Vti1 with or without Pra1.

They were incubated with Rab5/GDI, EEA1 and Rabenosyn5/hVps45 in the presence or absence of Rabaptin5/Rabex5 and hVps34/p150.

lane 1: Proteoliposome without Pra1.

Incubated with Rab5/GDI, Rabaptin5/Rabex5, hVps34/p150, EEA1 and Rabenosyn5/hVps45 (full-set)

lane 2: Pra1 proteoliposomes incubated with full-set.

lane 3: Pra1 proteoliposomes incubated with full-set without hVps34/p150

lane 4: HeLa endosomes incubated with full-set

lane 5: Pra1 proteoliposomes incubated with full-set without hVps34/p150

lane 6: Pra1 proteoliposomes incubated with full-set

lane 7: Pra1 proteoliposomes incubated with full-set without Rabaptin5/Rabex5

lane 8: Pra1 proteoliposomes incubated with full-set

lane 7: Old constructed

lane 8: Old constructed

lane 9: constructed in another methods

lane 10: constructed in another methods