

Appendix

Mammalian MemPrep establishes
the lipid composition of ER membranes in HEK293T cells

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Appendix Table S1: Total protein and total lipid yields

REEP5 MemPrep	Protein	Protein yield	Lipid	Lipid yield
whole cell lysate	12.3 ± 0.2 mg	100%	5.7 ± 2.3 μ mol	100%
P100.000	820 ± 97 μ g	6.7%	n.d.	n.d.
MemPrep isolate	16.2 ± 7.9 μ g	0.13%	68.8 ± 53.8 nmol	1.2%
SEC61β MemPrep	Protein	Protein yield	Lipid	Lipid yield
whole cell lysate	10.5 ± 1.5 mg	100%	4.9 ± 2.5 μ mol	100%
P100.000	977 ± 444 μ g	9.4%	n.d.	n.d.
MemPrep isolate	19.6 ± 2.9 μ g	0.19%	10.2 ± 6.7 nmol	0.2%

The total protein and lipid amounts are given as the mean of n = 3 replicates with the standard deviation. Note that the amount of lipids in the whole cell lysates and in the MemPrep insolates were determined in separate experiments.

Appendix Table S2: Oligonucleotides

Oligonucleotide	Sequence (5'-3')
REEP5-Tag fwd	AAGAGCACCGGGGGAGGCGGGGGTGGA
Tag-pBABE rv	GTCGACCACTGTGCTGGCGCTACTTGTTCATCGTCATCCTTGTAGTC GATGTCATGATCTTTATAATCACCGTC
pBABE-REEP5 fwd	CCAGTGTGGTGGTACGTAGGATGTCTGCGGCCATGAGG
REEP5-Tag rv	CGCCTCCCCCGGTGCTCTTCTTTCTTCACC
<i>Hind</i> III- _{pre} TAG fwd	AGAGAGAAGCTTGGGTCAGGTCTGGAAGTTCTGTTC
Sec61 β - <i>Eco</i> RI rv	AGAGAGGAATCCCTACGAACGAGT
<i>Xba</i> I-Sec61 β fwd	AGAGAGTCTAGAATGGACTACAAAGACC
Tag- <i>Kpn</i> I rv	AGAGAGGGTACCCTACGAACGAGT
pBABE-Tag fwd	CCAGTGTGGTGGTACGTAGGATGGACTACAAAGACCATG
Sec61 β -pBABE rv	GGTCGACCACTGTGCTGGCGCTACGAACGAGTGTACTTG

All oligonucleotides used for cloning are listed in a 5' to 3' direction.

Appendix Table S3: Plasmids

Plasmid	Source
pBABE	Martin van der Laan lab
pcDNA3.1	Invitrogen
pRE866	Ernst lab (Reinhard <i>et al</i> , 2024)
pUMVC Gag-Pol	Martin van der Laan lab
pCMV-VSV-G	Martin van der Laan lab
pMKRQ _{-pre} -TAP-tag-Sec61 β	Synthesized by Invitrogen
p3XFLAG-CMV-24	Sigma-Aldrich
p3XFLAG-HRV3C-Myc-Sec61 β -CMV-24	This study
pBABE-REEP5-Tag	This study
pBAEBE-Tag-Sec61 β	This study

Plasmids used in the study are listed together with the source.

Appendix Table S4: Primary sequence of bait constructs

Bait construct (size)	Sequence (single letter code)
REEP5-bait (241 aa)	MSAAMRERFDRFLHEKNCMTDLLAKLEAKTGVN RSFIALGVIGLVALYLVFGYGASLLCNLIGFGYPAY ISIKAIESPKNKEDDTQWLTYWVYGVFSIAEFFSDI FLSWFPFYMLKCGFLLWCMAPSPSNGAELLYK RIIRPFLLKHESQMDSVVKDLKDKAKETADAITKE AKKATVNLLGEEKKSTGGGGGGG EQKLISEEDL G SG LEVLFG PGSG DYKDHDGDYKDHDIDYKDD DK
bait-Sec61 β (152 aa)	MDYKDHDGDYKDHDIDYKDDDDK LGSG LEVLFG PGSGEQKLISEEDL GGGGGGASMPGPTPSGT NVGSSGRSPSKAVAARAAGSTVRQRKNASCGTR SAGRTTSAGTGGMWRFYTEDSPGLKVGPPVPLV MSLLFIASVFMLHIWGKYTRS

Primary amino acid sequence of the bait proteins used in the study. Highlighted sequence elements: **3xFLAG tag**, **3C protease recognition site**, and **myc tag**.

Appendix Table S5: Antibodies for immunoblotting (IB) and immuno-fluorescence (IF).

Antibody	Dilution	Source	RRID / [clone]
Mouse α -GAPDH	IB: 1:1000	Abcam (ab8245)	AB_2107448
Rabbit α -REEP5	IB: 1:1000	Abcam (ab186755)	[EP11115]
Rabbit α -SEC61B	IB: 1:1000	Proteintech (15087-1-AP)	AB_2186411
Rabbit α -Rtn4	IF 1:500	Abcam (ab47085)	AB_881718
Rabbit α -CLIMP-63	IF 1:500	Abcam (ab302539)	[EPR25713-62]
Rabbit anti-TOMM22	IB: 1:1000	Abcam (ab179826)	AB_3095411
Rabbit α -Histone H3	IB: 1:1000	Cell Signaling (9715S)	AB_331563
Rabbit α -Calnexin	IB: 1:1000 IF: 1:500	Proteintech (10427-2-AP)	AB_2069033
Mouse α -SERCA2	IB: 1:1000	Invitrogen (MA3-919)	AB_325502
Rabbit α -Na/K-ATPase	IB: 1:1000	Abcam (ab76020)	AB_1310695
Rabbit α -EEA1	IB: 1:1000	Abcam (ab109110)	AB_10863524
Mouse α -FLAG	IB: 1:1000 IF: 1:500	Sigma-Aldrich (F1804)	AB_262044
Goat α -Mouse IgG IRDye 800CW	IB: 1:2000	LI-COR Biosci. (926-32210)	AB_621842
Goat α -Rabbit IgG IRDye 800CW	IB: 1:2000	LI-COR Biosci. (926-32211)	AB_621843
Goat α -Mouse IgG Alexa Fluor™ 488	IF: 1:500	ThermoFisher (A11029)	AB_2534088
Goat α -Rabbit IgG Alexa Fluor™ 555	IF: 1:500	ThermoFisher (A21428)	AB_2535849

Antibodies used for immunoblotting and immunofluorescence are listed together with their source.

Appendix Supplementary Text: HelixHarbor Docker Installation Guide

This guide provides instructions to download, run, and update the HelixHarbor Docker container. Ensure Docker is installed on your system. If not, follow the official Docker installation guide.

Download and Run Docker Container

1. Pull the latest version of the Docker image:

```
docker pull mmofty/helixharbor:versionx.x
```

This command downloads the latest version of the image from Docker Hub.

2. Run the Docker container with the following command:

```
docker run --restart=always -d -p 5005:5005 mmofty/helixharbor:versionx.x
```

This command does the following:

- `restart=always`: Ensures the container restarts automatically if it crashes or if the Docker daemon restarts.
- `-d`: Runs the container in detached mode.
- `-p 5005:5005`: Maps port 5005 on your host to port 5005 in the container.
- `mmofty/helixharbor:versionx.x`: Specifies the 'x.x' version of the Docker image to use.

3. Verify that the container is running:

```
docker ps
```

You should see an entry for the `mmofty/helixharbor:latest` container in the output.

Update to the Latest Version

1. Pull the latest version of the Docker image:

```
docker pull mmofty/helixharbor:latest
```

2. List the running containers to find the container ID:

```
docker ps
```

Note the container ID for the currently running `mmofty/helixharbor` container.

3. Stop and remove the existing container:

```
docker stop <container_id> && docker rm <container_id>
```

Replace `<container_id>` with the actual container ID you obtained from the previous step.

4. Run the updated Docker container:

```
docker run --restart=always -d -p 5005:5005 mmofty/helixharbor:latest
```

5. Verify that the updated container is running:

```
docker ps
```

You should see an entry for the `mmofty/helixharbor:latest` container in the output.

Additional Tips

Check Logs: If you encounter any issues, you can check the container logs with:

```
docker logs <container_id>
```

Stop Container: To stop the running container:

```
docker stop <container_id>
```

Remove Container: To remove a stopped container:

```
docker rm <container_id>
```

List All Containers: To see all containers (running and stopped):

```
docker ps -a
```

Remove Unused Images: To free up space by removing unused Docker images:

```
docker image prune -a
```

Appendix References

Reinhard J, Starke L, Klose C, Haberkant P, Hammarén H, Stein F, Klein O, Berhorst C, Stumpf H, Sáenz JP, *et al* (2024) MemPrep, a new technology for isolating organellar membranes provides fingerprints of lipid bilayer stress. EMBO J 43: 1653–1685