



Transcription Factors:

Optimized LOV domain: hLOV evolved from *Avena sativa* (PMID 29189201)

Mitochondrial transcription factor (Figures 1-4, 6)

Mito TMD LOV TEVcs TF (GAL4dbd-VP64) V5

↓ From AKAP1

MAIQLRSLFPLALPGLLALLGWWWWFFSRKK

ER transcription factor (Figures 1-4, 6)

ER targeting domain LOV TEVcs TF (GAL4dbd-VP64) V5

↓ Full-length TMED3

MGSTVPRASVLLLLLLRR ... VLLKSFTEKRPISRAVHS

Peroxisomal transcription factor (Figure 1 - figure supplement 4)

Peroxisome targeting domain LOV TEVcs TF (GAL4dbd-VP64) V5

↓ Full-length PMP34

MASVLSYESLVHAVAGAVGS ... YEKLTAATFTVMGLKRAHQH

Proteases:

Optimized protease: uTEV1Δ evolved from Tobacco etch virus (PMID 31819267)

"TA protease" - tail-anchored protease (Figures 1-4, 6)

EGFP TEV protease Mito TMD

↓ From MAVS

RPSPGALWLQVAVTGVLVVTLLVLYRRLH

"mTA\* protease" - mutant tail-anchored protease (Figures 2-4, 6,)

EGFP TEV protease Mito TMD

↓ From MAVS

RPSPGALWLQVAVTGVLVVTLLVLYR<sup>AR</sup>LH

"SA protease" - signal-anchored protease (Figures 2-4, 6)

Mito TMD EGFP TEV protease

↓ From AKAP1

MAIQLRSLFPLALPGLLALLGWWWWFFSRKK

ER protease (Figure 1)

EGFP TEV protease ER TMD

↓ From CYB5A

ITTVESNSSWWTNWVIPAISALVVALMYRLYMAED

Cytoplasmic protease (Figure 1)

EGFP TEV protease NES

↓

ELA<sup>E</sup>EKL<sup>E</sup>AGLDIN

Peroxisomal protease (Figure 1 - figure supplement 4)

EGFP TEV protease PO TMD

↓ From ACBD5

FEMSPGVLTF<sup>E</sup>AIWPFIAQWL<sup>E</sup>VYLYYQRRRRKLN

EMC client (SQS-tmd) protease (Figure 6 - figure supplement)

EGFP TEV protease ER TMD

↓

CQLISRSHYSPIYLSFVMLLAALSWQYLTTLSQVTE<sup>E</sup>DYVQTGEH

EMC client (SQS-FL) protease (Figure 6 - figure supplement)

EGFP TEV protease ER targeting domain

Full length SQS ↓

EFVKCLGHPEEFYNLVR<sup>E</sup>FRI ... QYLTTLSQVTE<sup>E</sup>DYVQTGEH