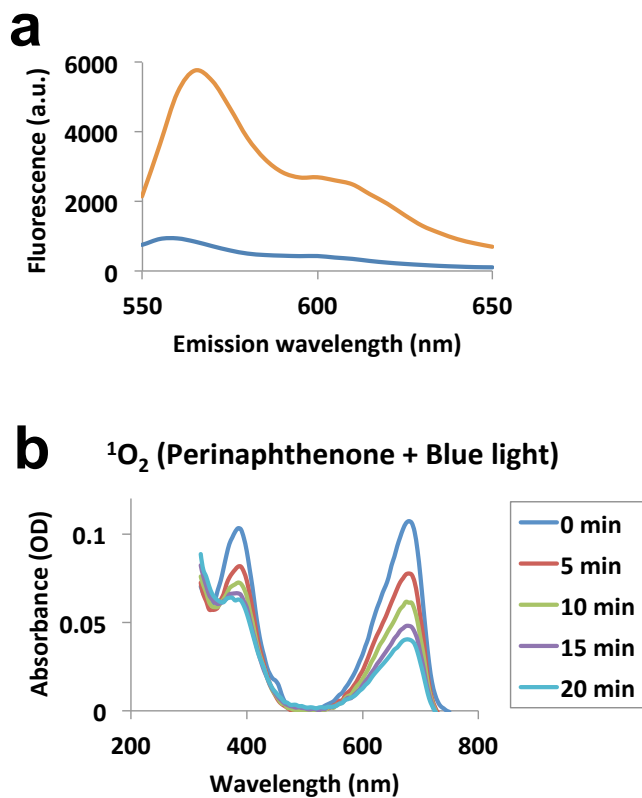
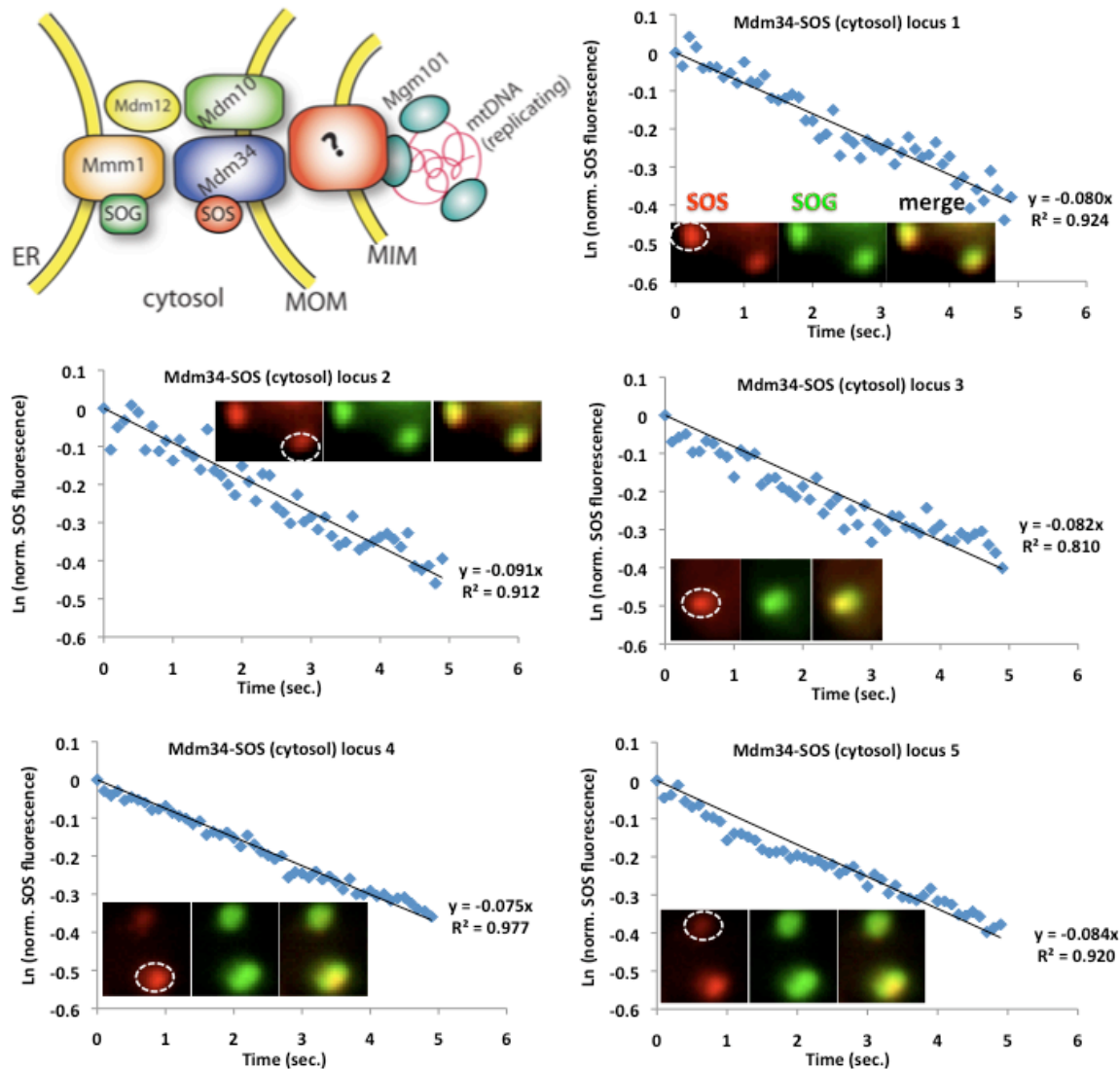


## Supplementary Figures.

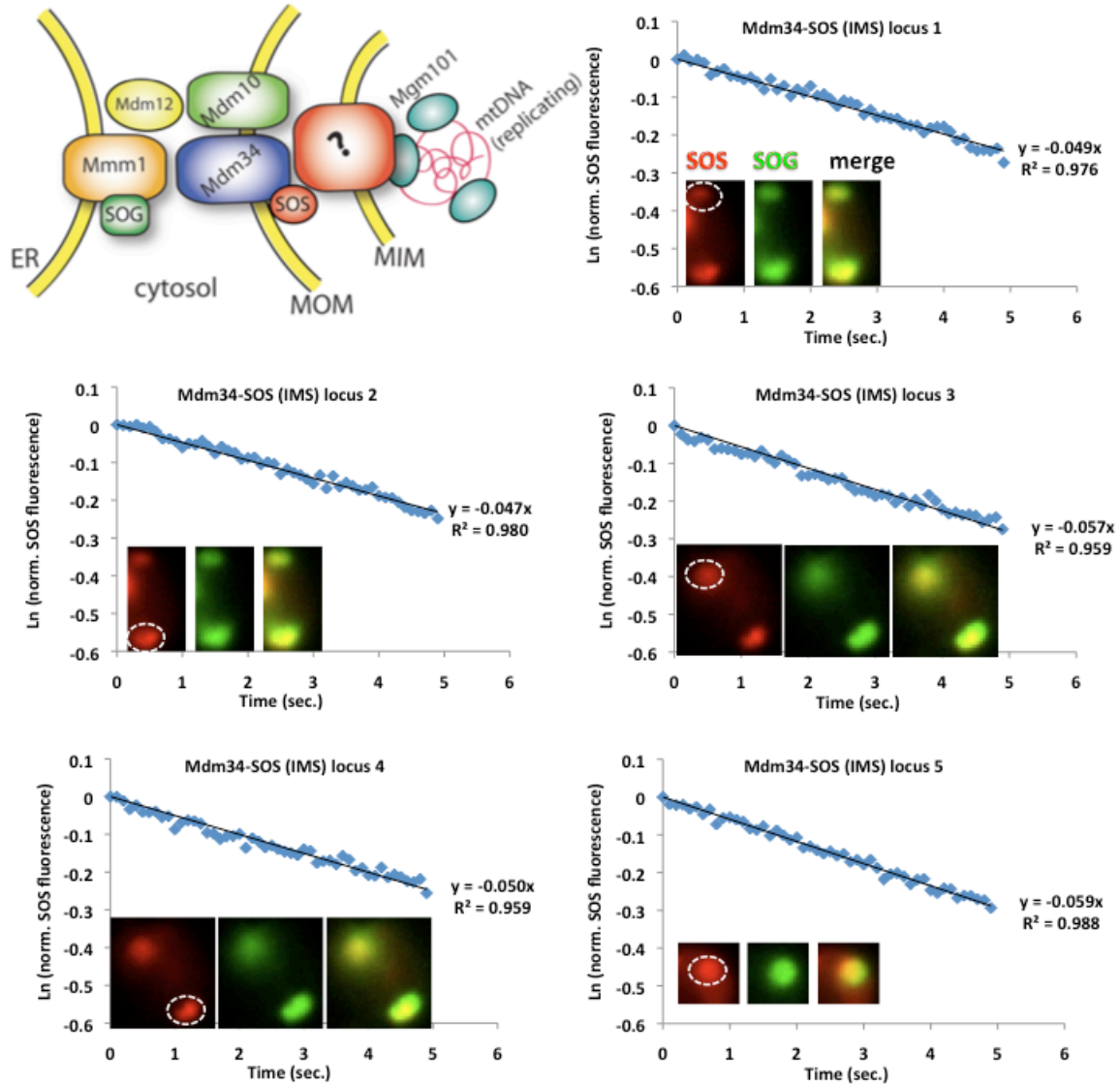


**Supplementary Figure 1. Reaction of hydro-cyanine 3 with superoxide and reaction of IFP1.4 with singlet oxygen.** (a) Blue line: fluorescence spectra of 50  $\mu\text{M}$  hydrocyanine 3; Orange line: fluorescence spectra of 50  $\mu\text{M}$  hydrocyanine 3 upon reaction with 1 mM  $\text{KO}_2$ . (b) Absorbance spectra of 5  $\mu\text{M}$  IFP1.4 reacted with 10  $\mu\text{M}$  Perinaththenone upon blue light excitation.

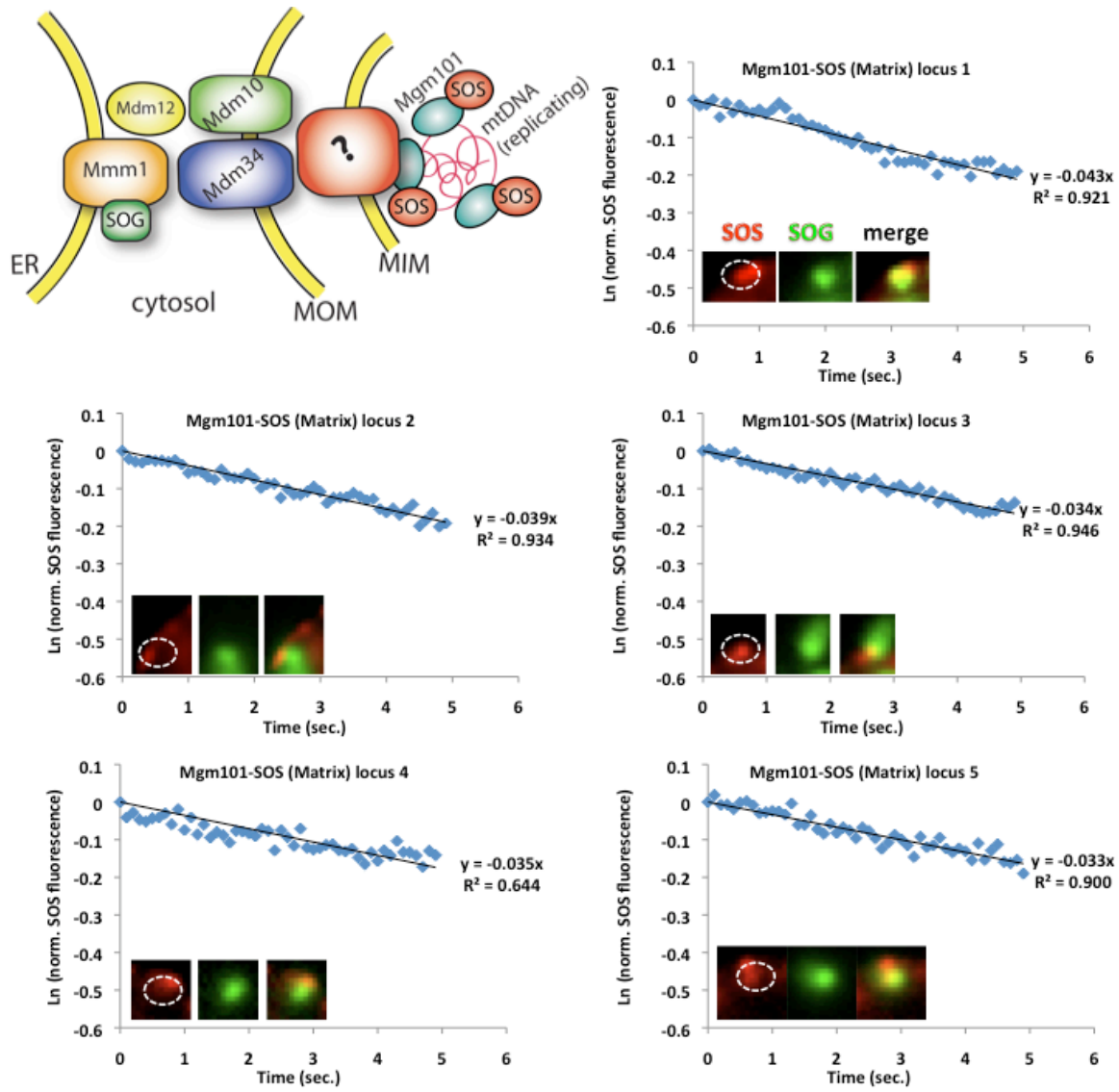


**Supplementary Figure 2. STET from Mmm1 to the cytosolic face of Mdm34.**

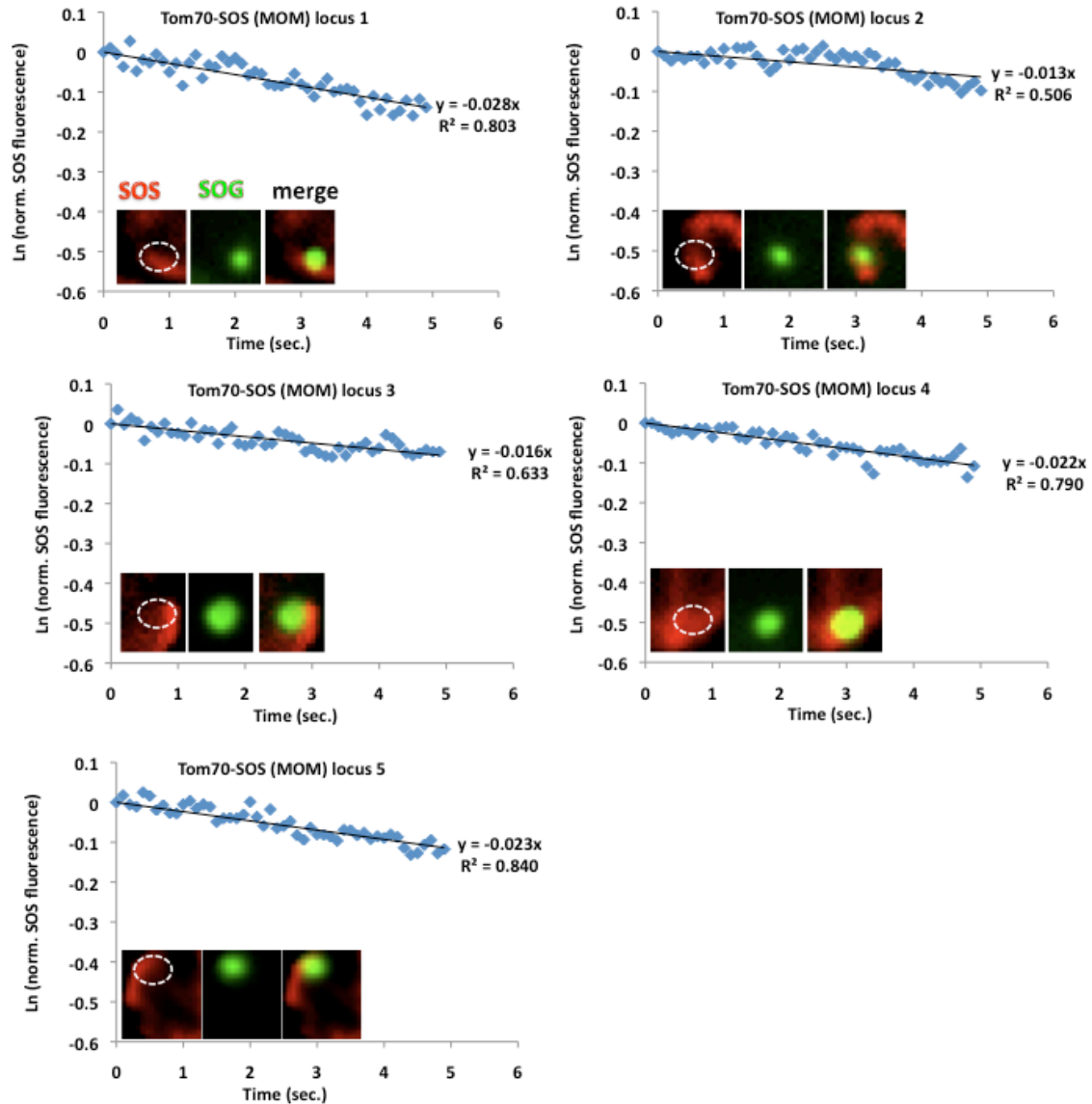
Schematic diagram showing topology of the ERMES complex with SOG and SOS attached to the cytosolic face of Mmm1 and Mdm34, respectively. SOS is fused to the C-terminus of Mdm34. Natural logarithm of normalized SOS fluorescence intensity (white circle) versus illumination time of blue light is plotted for 5 individual loci. [Blue diamond: measured data point. Solid line: linear fit of the data.]



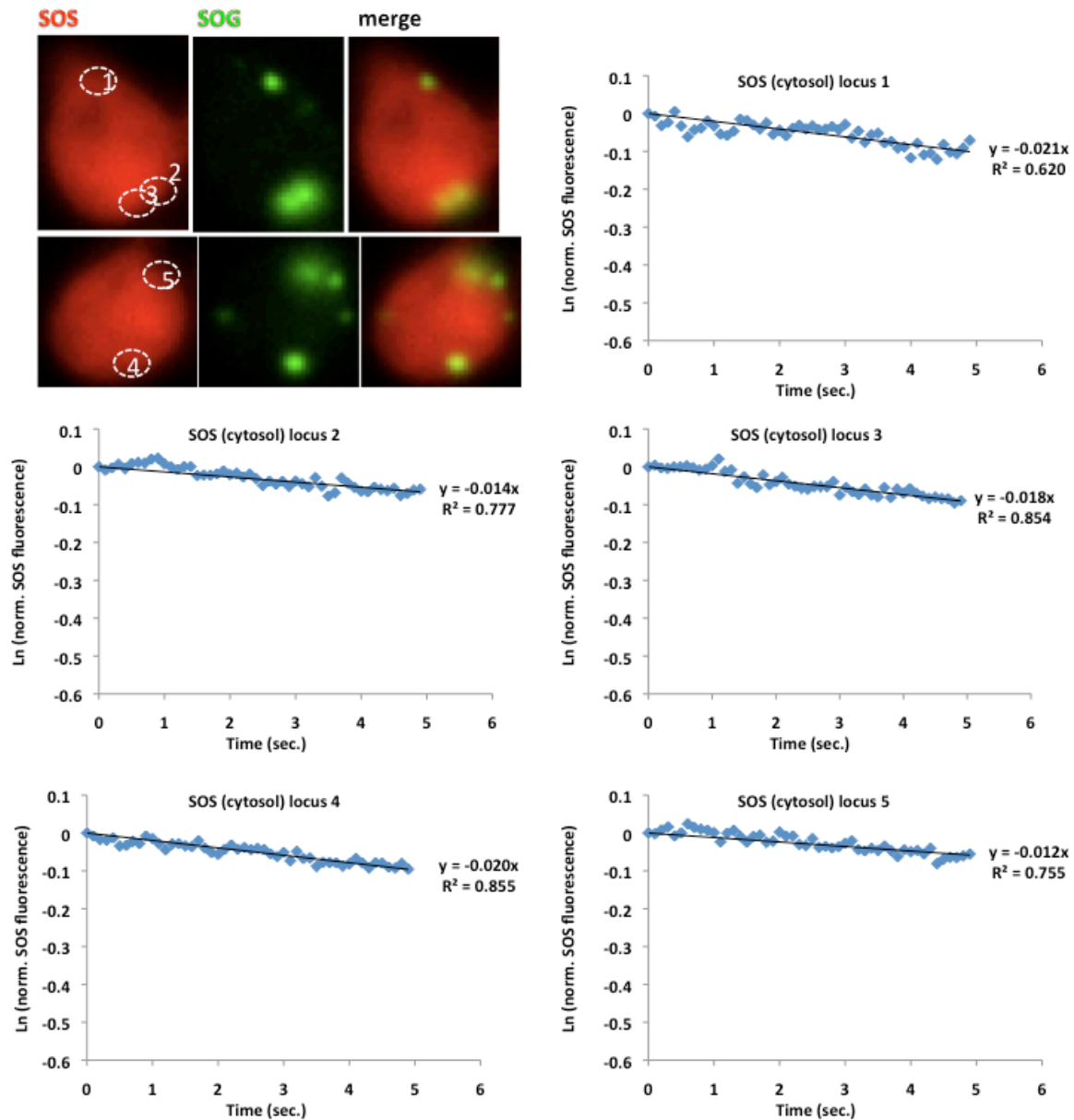
**Supplementary Figure 3. STET from Mmm1 to the IMS face of Mdm34.** Schematic diagram showing topology of the ERMES complex with SOG attached to cytosolic face of Mmm1 and SOS attached to the IMS face of Mdm34. SOS is fused to the N-terminus of Mdm34. Natural logarithm of normalized SOS fluorescence intensity (white circle) versus illumination time of blue light is plotted for 5 individual loci. [Blue diamond: measured data point. Solid line: linear fit of the data.]



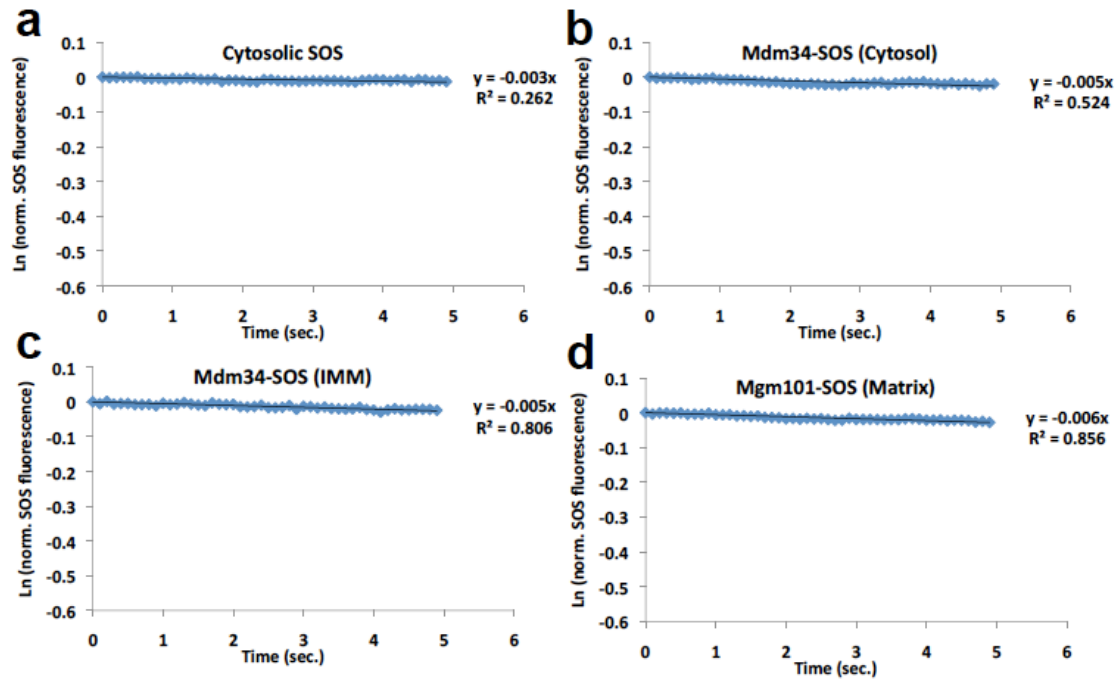
**Supplementary Figure 4. STET from Mmm1 to Mgm101.** Schematic diagram showing topology of the ERMES complex with SOG attached to the cytosolic face of Mmm1 and SOS to Mgm101 in the mitochondrial matrix. Natural logarithm of normalized SOS fluorescence intensity (overlapped region, indicated by the white circle) versus illumination time of blue light is plotted for 5 individual loci. [Blue diamond: measured data point. Solid line: linear fit of the data.]



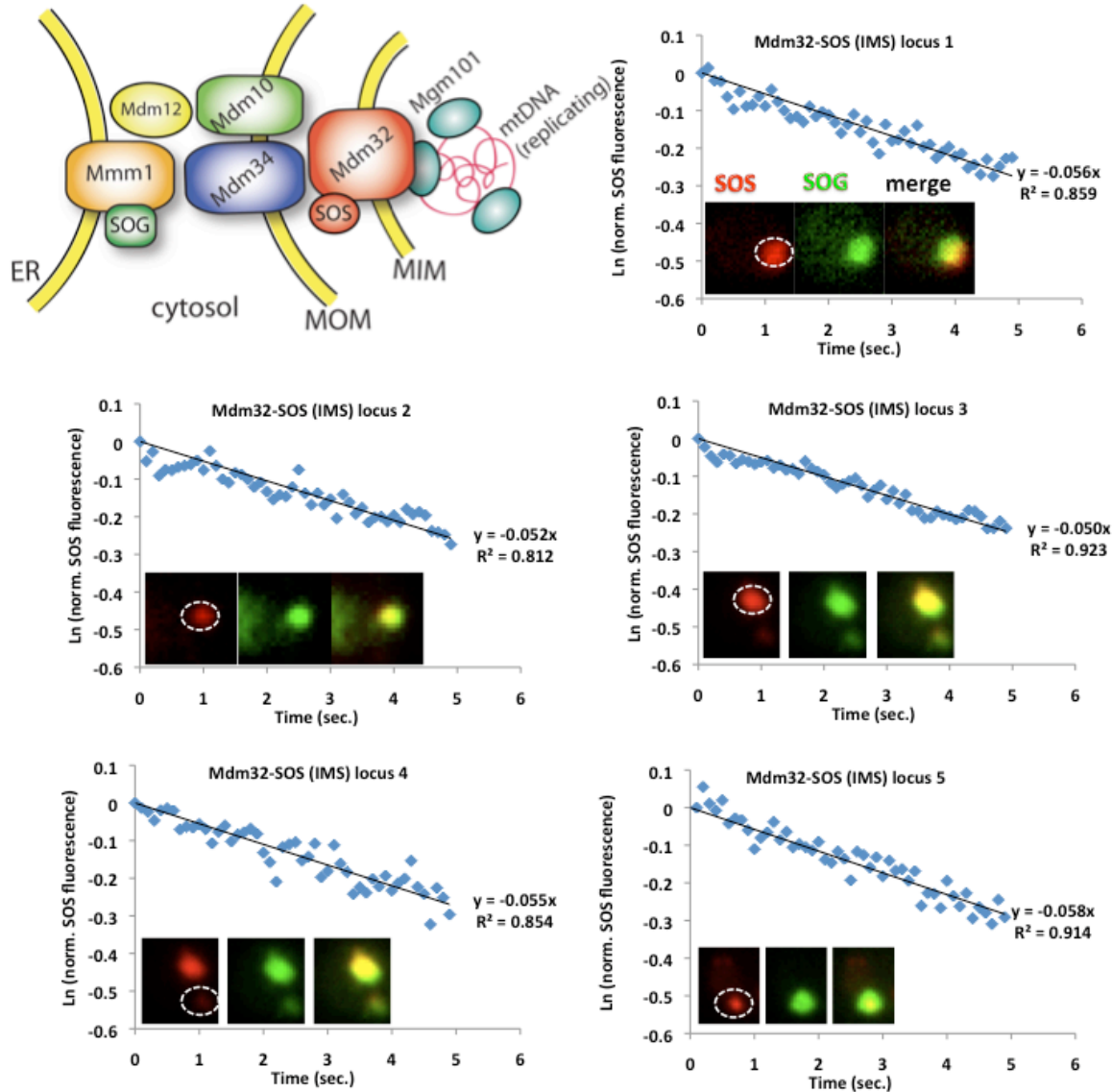
**Supplementary Figure 5. STET from Mmm1 to the cytosolic face of Tom70 (non-interacting).** STET experiment was performed with SOG attached to the cytosolic face of Mmm1 and SOS to the cytosolic face of Tom70. Natural logarithm of normalized SOS fluorescence intensity (overlapped region, indicated by the white circle) versus illumination time of blue light is plotted for 5 individual loci. [Blue diamond: measured data point. Solid line: linear fit of the data.]



**Supplementary Figure 6. STET from Mmm1 to free cytosolic protein (non-interacting).** STET experiment was performed with SOG attached to the cytosolic face of Mmm1 and free SOS. Natural logarithm of normalized SOS fluorescence intensity (overlapped region, indicated by the white circle) versus illumination time of blue light is plotted for 5 individual loci. [Blue diamond: measured data point. Solid line: linear fit of the data.]

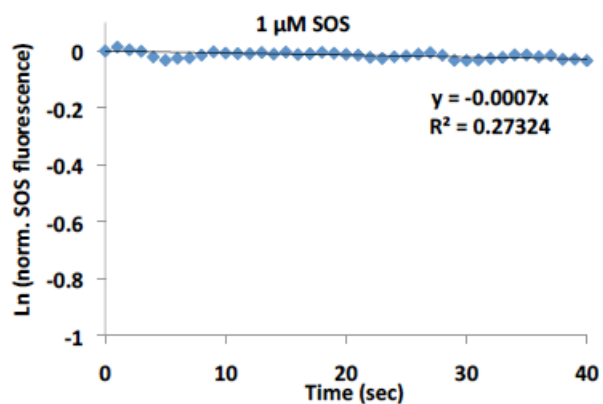


**Supplementary Figure 7. Negligible change of SOS fluorescence in the absence of SOG.** (A) Free SOS and SOS attached to the (B) cytosolic face of Mdm34; (C) IMS face of Mdm34; and (D) Mgm101 in the matrix were illuminated under the same conditions as in **Supplementary Figures 2-6**. In (A–D), the natural logarithm of normalized SOS fluorescence is plotted against the illumination time of blue light. [Blue diamond: measured data point. Solid line: linear fit of the data.]

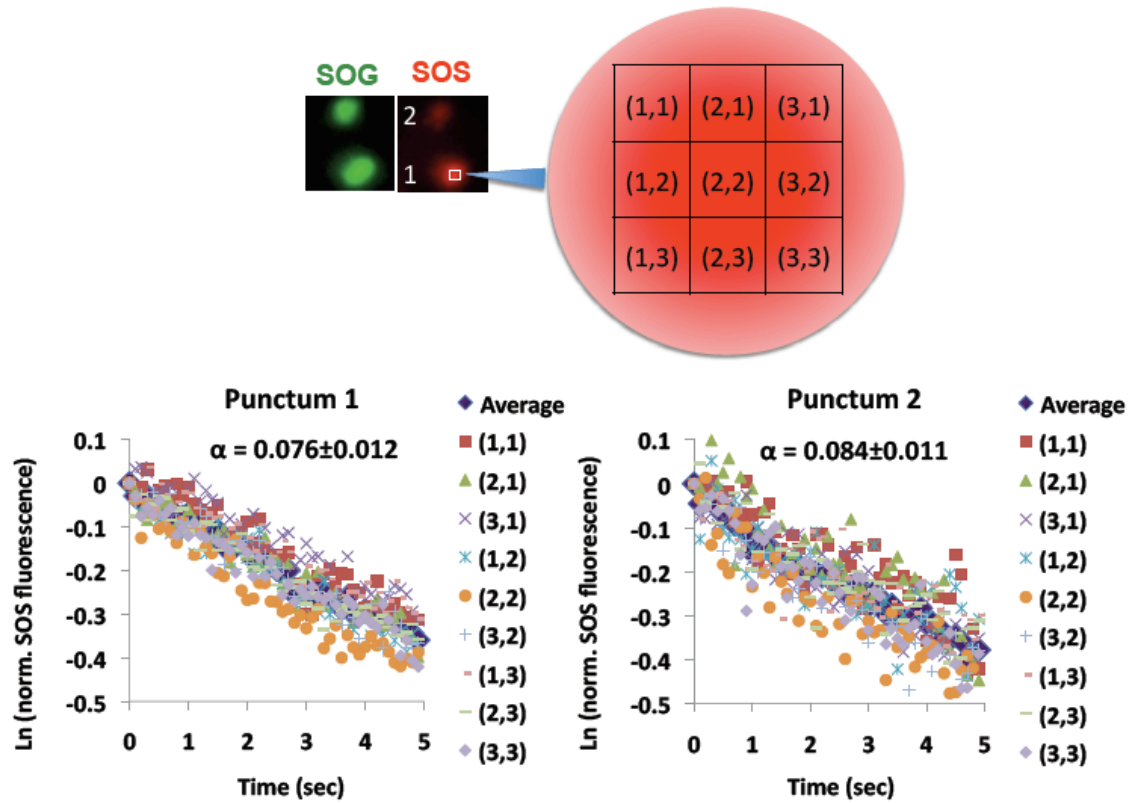


**Supplementary Figure 8. STET from Mmm1 to Mdm32.** Schematic diagram showing topology of the ERMES complex with SOG attached to cytosolic face of Mmm1 and SOS attached to the IMS face of Mdm32. SOS is fused to the C-terminus of Mdm32. Natural logarithm of normalized SOS fluorescence intensity (white circle) versus illumination time of blue light is plotted for 5 individual loci. [Blue diamond: measured data point. Solid line: linear fit of the data.]





**Supplementary Figure 9. Negligible change of SOS fluorescence in the absence of SOG.** The natural logarithm of normalized SOS fluorescence is plotted against the illumination time of blue light. The same illumination condition as in **Figure 1b** was used. [Blue diamond: measured data point. Solid line: linear fit of the data.]



**Supplementary Figure 10. Pixel-to-pixel variation in STET signals within a punctum is small.** The two punctate structures analyzed are identical to locus 4 and locus 5 in **Figure S1** (Mmm1-SOG and Mdm34-SOS). Natural logarithm of normalized SOS fluorescence intensity versus illumination time of blue light is plotted for 9 individual pixels (center and its 8 immediate neighbors in a 3x3 region) in a single punctum. The center (2, 2) is the pixel with the largest fluorescence loss. The STET signals are determined for individual pixels and the standard deviation in STET is ~15% over the 3x3 region.

## Supplementary Tables.

**Supplementary Table 1.** List of plasmids used in the study

Vector ID	Description
pTLT049	p426 TEF MMM1-miniSOG
pTLT120	p424 TEF MDM34-IFP1.4
pTLT124	p424 TEF IFP1.4
pTLT126	p424 TEF TOM70-IFP1.4
pTLT141	p414 TEF MGM101-IFP1.4
pTLT153	p424 TEF IFP1.4-MDM34
pTLT155	p424 TEF MDM32-IFP1.4
pTLT178	pBAD IFP1.4-HO1
pTLT181	PBAD IFP1.4-5aa-miniSOG-HO1
pTLT182	pBAD miniSOG
pTLT184	pRS303 TEF HO1
pTLT390	pBAD IFP1.4-73aa-miniSOG-HO1
pTLT393	pBAD IFP1.4-133aa-miniSOG-HO1