

Insights on autophagosome-lysosome tethering from structural and biochemical characterization of human autophagy factor EPG5

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Supplementary Information

Supplementary Figure 1. Purification of the hEPG5 constructs.

Supplementary Figure 2. *In vitro* pulldown assays of hEPG5 mutants and the LC3/GABARAP subfamily.

Supplementary Figure 3. Comparison of the LIR2-GABARAPL1 crystal structure with previously published apo- and LIR bound-GABARAPL1 crystal structures.

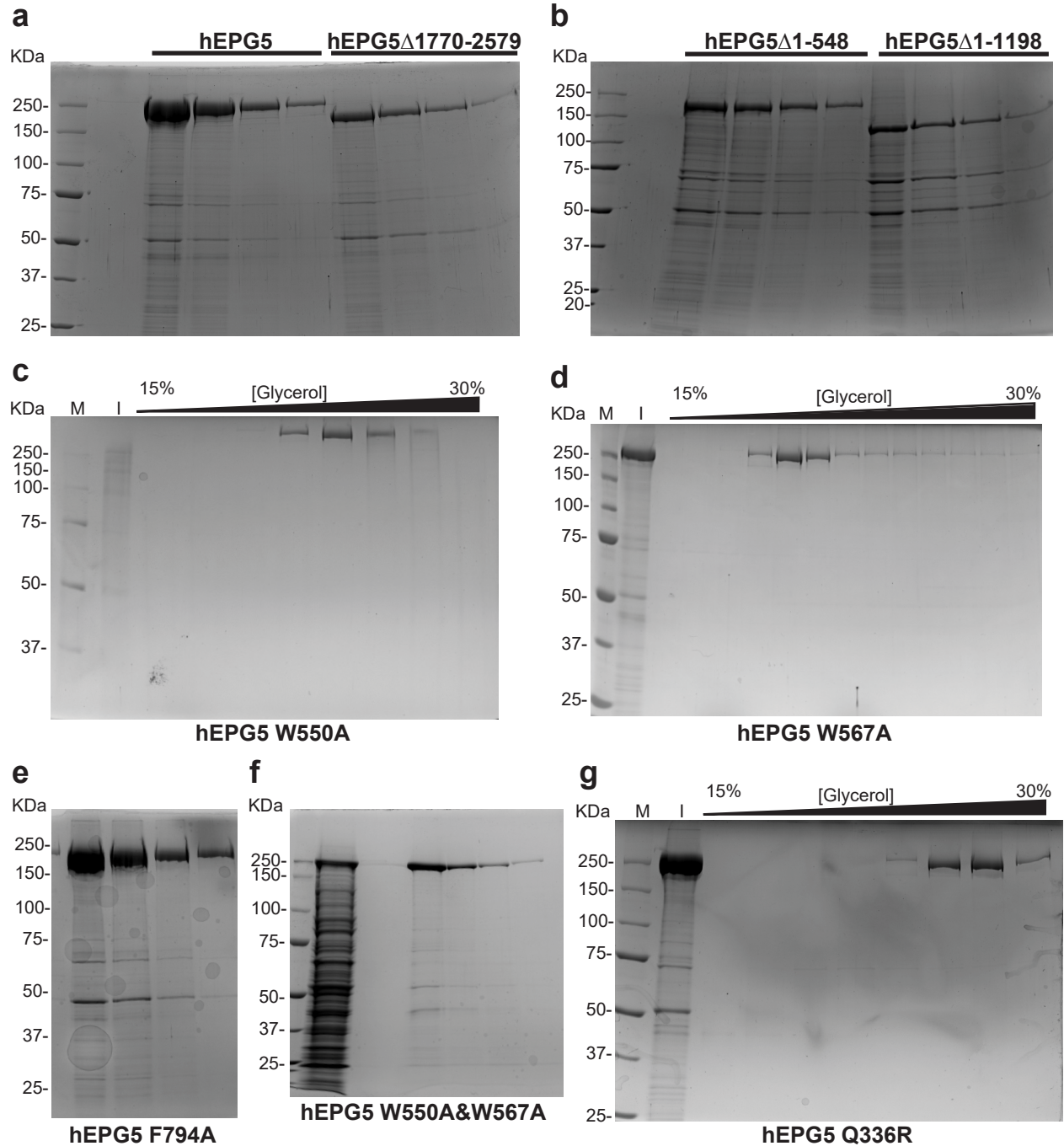
Supplementary Figure 4. Gold-standard Fourier shell correlation curve.

Supplementary Figure 5. Uncropped blots for Figure 2 and 6

Supplementary Figure 6. Uncropped blots for Supplementary Figure 2

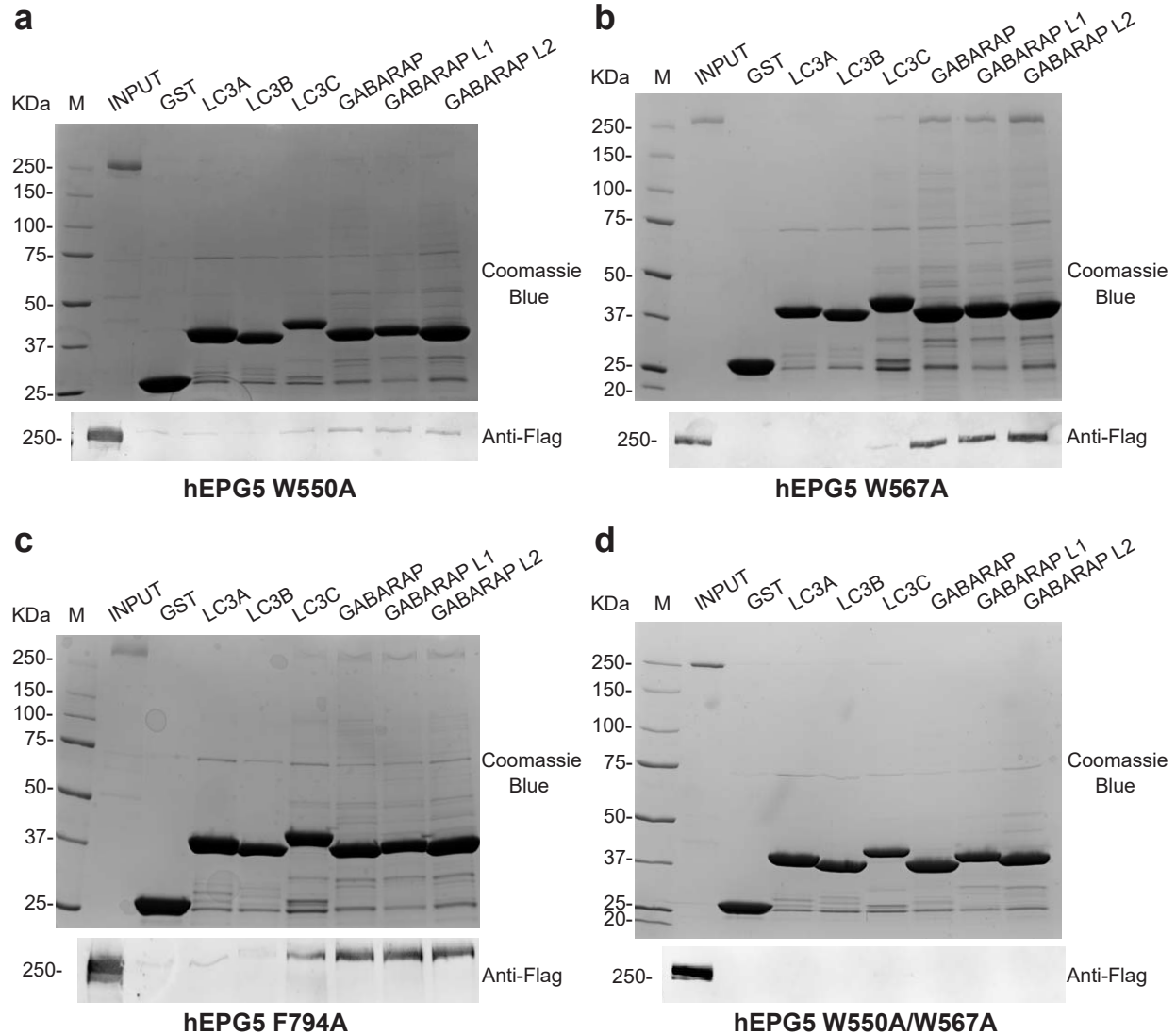
Supplementary Table 1. Thermodynamic parameters of ITC experiments of human ATG8 proteins with hEPG5-LIR1 and LIR2 peptides.

Supplementary Table 2. Negative stain EM datasets and 2D analysis details.



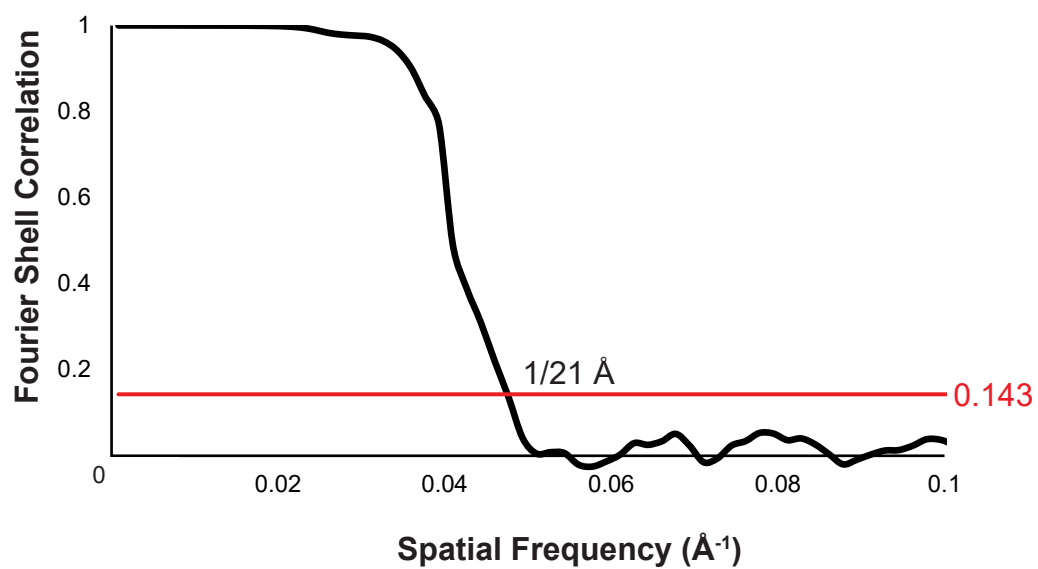
Supplementary Figure 1. Purification of the hEPG5 constructs. a-g 6%-15% SDS-PAGE of His-FLAG-hEPG5 mutants isolated by anti-FLAG affinity chromatography and glycerol gradient ultracentrifugation fractions, stained with Coomassie Blue. M and I represent the protein marker and input, respectively. **(a)** Elution fractions of wild type hEPG5 and C-terminal truncation mutant hEPG5 Δ 1770-2579. **(b)** Elution fractions of hEPG5 N-terminal truncation mutant hEPG5 Δ 1-548 and hEPG5 Δ 1-1198. **(c)** hEPG5 mutant with LIR1 aromatic residue Trp550 mutated to alanine. **(d)** hEPG5 mutant with LIR2 aromatic residue Trp567

mutated to alanine. **(e)** Elution fractions of hEPG5 mutant with LIR3 aromatic residue Phe794 mutated to alanine. **(f)** Elution fractions of hEPG5 double mutant with both LIR1 aromatic residue Trp550 and LIR2 aromatic residue Trp567 mutated to alanine. **(g)** hEPG5 Vici syndrome mutant with residue Gln336 mutated to arginine.



Supplementary Figure 2. *In vitro* pulldown assays of hEPG5 mutants and the LC3/GABARAP subfamily. a-d *In vitro* pulldown assays of hEPG5 mutants with GST (control) and GST-tagged LC3/GABARAP subfamily proteins used as baits. Representative 6%-15% SDS-PAGE stained with Coomassie Blue (top panel) shows the hEPG5 mutants and GST-tagged LC3/GABARAP input, and representative Western blot (bottom panel) was probed by anti-FLAG antibodies. M represents the protein marker. Experiments were performed in triplicates. **(a)** hEPG5^{W550A} mutant was used as prey. **(b)** hEPG5^{W567A} mutant was used as prey. **(c)** hEPG5^{F794A} mutant was used as prey. **(d)** hEPG5^{W550A/W567A} mutant was used as prey.

GABARAPL1 (orange; PDB:2R2Q). Residue Lys46 sidechain shifts outwards by 8.0 Å upon LIR2 binding. **e** Close-up view apo-GABARAPL1. Ribbon and transparent surface representation of apo-GABARAPL1 (orange; PDB:2R2Q), with residue Lys46 highlighted in green. **f** Close-up view LIR2 bound-GABARAPL1. Ribbon and transparent surface representation of GABARAPL1 (grey) with LIR2 (pink) bound. Residue Lys46 is highlighted in green. The conformational rearrangement of Lys46 creates space to accommodate LIR2 residue I568 binding.



Supplementary Figure 4. Gold-standard Fourier shell correlation curve. The estimated resolution of the refined hEPG5 3D reconstruction is 21 Å using the 0.143 criterion.

Fig. 2a

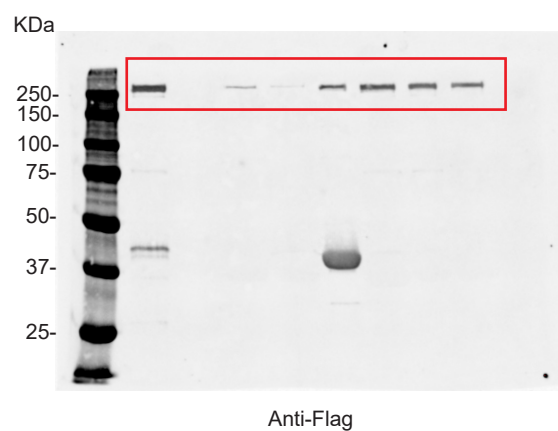


Fig. 2c

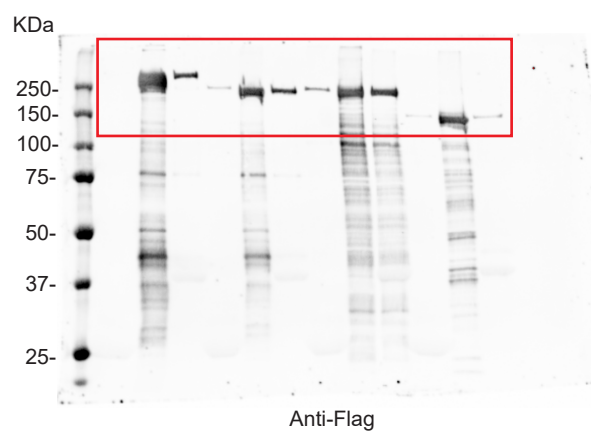


Fig. 2e

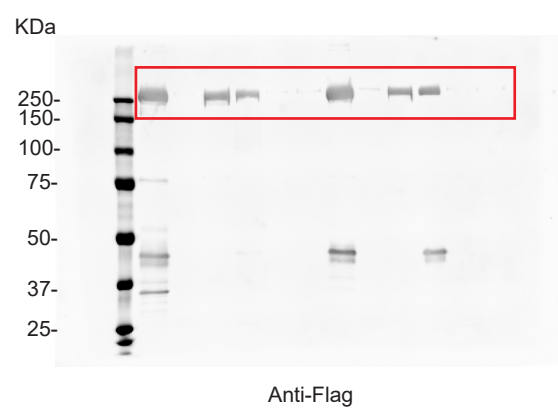
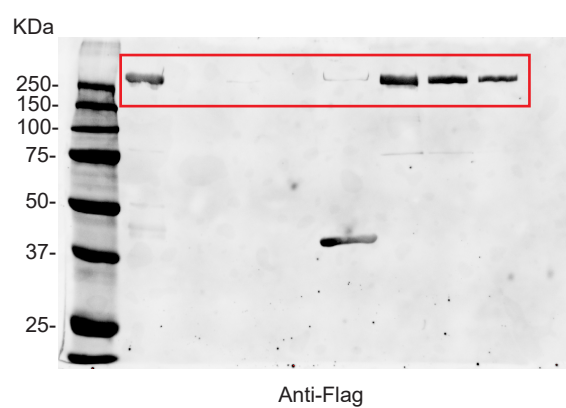
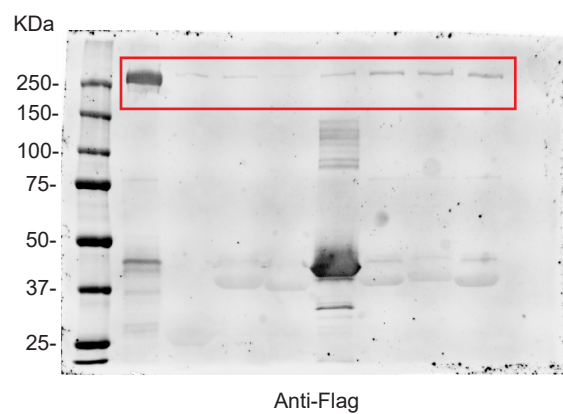


Fig. 6d

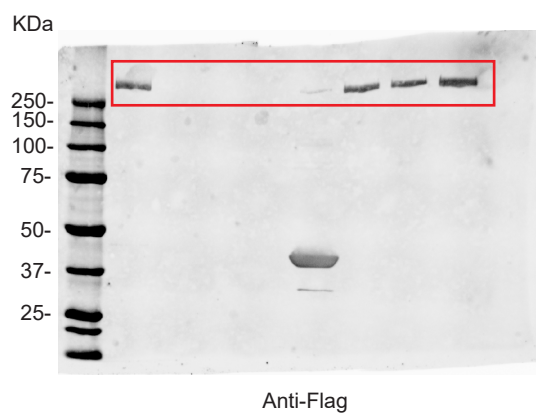


Supplementary Figure 5 | Uncropped blots for Figure 2 and 6.

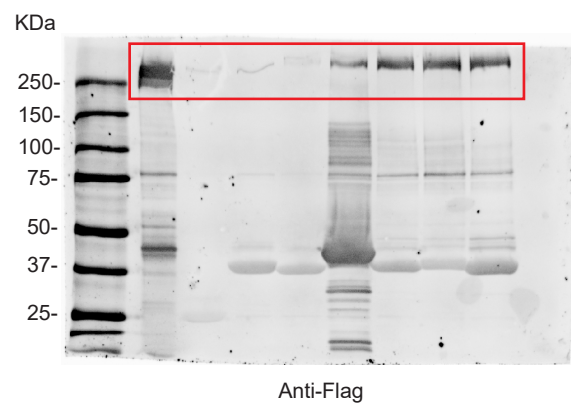
Supplementary Fig. 2a



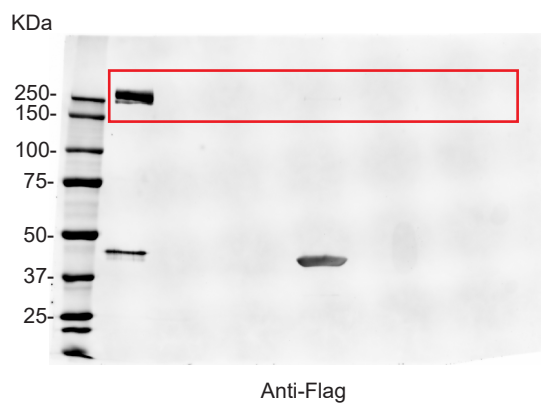
Supplementary Fig. 2b



Supplementary Fig. 2c



Supplementary Fig. 2d



Supplementary Figure 6 | Uncropped blots for Supplementary Figure 2.

Supplementary Table 1. Thermodynamic parameters of Isothermal Titration Calorimetry (ITC) experiments of human ATG8 proteins with hEPG5-LIR1 and LIR2 peptides.

	ΔH (kcal mol ⁻¹)	ΔS (cal mol ⁻¹ K ⁻¹)	ΔG (kcal mol ⁻¹)	K_A (x10 ⁶ M ⁻¹)	K_D (μ M)	N
hEPG5-LIR1 (GSGTWTLVDEG)						
LC3A	-4.7 $\pm$ 0.7	5.55	-6.40	0.05 $\pm$ 0.01	20.38	1.06 $\pm$ 0.16
LC3B	No Binding Determinable					
LC3C	No Binding Determinable					
GABARAP	-6.8 $\pm$ 0.4	0.65	-7.00	0.14 $\pm$ 0.03	7.47	0.96 $\pm$ 0.09
GABARAPL1	-6.1 $\pm$ 0.4	2.78	-6.92	0.12 $\pm$ 0.01	8.54	0.85 $\pm$ 0.22
GABARAPL2	-4.2 $\pm$ 0.4	8.68	-6.73	0.09 $\pm$ 0.02	11.79	0.94 $\pm$ 0.02
hEPG5-LIR2 (DEDPETSWILLN)						
LC3A	-3.6 $\pm$ 0.1	14.4	-7.87	0.58 $\pm$ 0.10	1.75	0.93 $\pm$ 0.03
LC3B	-3.5 $\pm$ 0.3	13.1	-7.36	0.25 $\pm$ 0.02	4.07	0.94 $\pm$ 0.05
LC3C	No Binding Determinable					
GABARAP	-5.6 $\pm$ 0.2	12.3	-9.30	6.79 $\pm$ 2.42	0.16	0.93 $\pm$ 0.04
GABARAPL1	-5.2 $\pm$ 0.4	14.8	-9.64	11.77 $\pm$ 0.72	0.09	0.90 $\pm$ 0.01
GABARAPL2	-2.0 $\pm$ 0.2	21.6	-6.44	1.57 $\pm$ 0.47	0.68	0.84 $\pm$ 0.04

Supplementary Table 2. Negative stain EM datasets and 2D analysis details.

Protein/Protein complex	Total number of particles	Number of 2D classes specified	Number of particles in representative image
hEPG5 (Fig. 1d)	10,866	50	1,581
hEPG5^{Δ2079-2579} (Fig. 1e)	127	4	48
hEPG5-MBP (Fig. 1f)	3,167	25	62
hEPG5-GABARAP (Fig. 2d)	3,253	18	279
hEPG5^{Q336R} (Fig. 6a)	6,574	15	778

Supplementary References

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