

## Fig. S1. Co-staining for organelle markers and myc-tagged DmPIG-N, -F, or -O in S2 cells

- (A) Immunofluorescence analysis of DmPIG proteins and Calreticulin (Calr-GFP, ER marker) in S2 cells expressing myc-tagged DmPIG proteins (DmPIG-N, -F, and -O) and Calr-GFP. Myc-tagged DmPIG proteins were stained with anti-myc antibody (magenta) and the ER was labeled with anti-GFP antibody (green). Arrows indicate co-localization of DmPIG proteins with Calr-GFP. Bar, 5 μm.
- (B) Immunofluorescence analysis of DmPIG proteins and Rab7 (late endosome marker) in S2 cells expressing myc-tagged DmPIG proteins (DmPIG-N, -F, and -O). Myc-tagged DmPIG proteins were stained with anti-myc antibody (magenta) and late endosomes were labeled with anti-Rab7 antibody (green). Arrows indicate Rab7-positive endosomes and arrowheads indicate DmPIG proteins. Bar, 5 μm.



## Fig. S2. Low expression level and rescue efficiency of human PIG-B in Drosophila

- (A) Immunoblot analysis of myc-tagged DmPIG-B (Dm) and human PIG-B (human) expressed in third instar larvae under the control of da-Gal4. Proteins were detected with anti-myc and anti-tubulin (loading control) antibodies.
- (B) Rescue of *PIG-B*<sup>13</sup> lethality by expression of myc-tagged DmPIG-B (Dm) and human PIG-B (human) under the control of da-Gal4 at 25° C. Values are means  $\pm$  S.D. of data obtained in three independent crosses; more than 100 adults were scored for each cross.

Ant	FRFILPLLPL-LICTSFT	SL	KYLNINAN	VRKRIIAM	FMC <mark>SNI</mark>	II <mark>GL</mark> FISM <mark>IH</mark>
Honeybee	FRFLLPLLPMCIY <mark>IC</mark> TSCT	FH	LNM <mark>K</mark> FMKTA	ARKIFVGF	LIL <mark>S</mark> NV	L <mark>PGLY</mark> FN <mark>LVH</mark>
Drosophila	FRFVS <mark>PLLP</mark> LCLYVITDAL	SR	W <mark>S</mark> IRASSTN	M <mark>LW</mark> TT <mark>A</mark> LV	ILV <mark>G</mark> NV	MPAWYLST <mark>VH</mark>
Mosquito	FRFLLPVLPMCLFITADYL	AR	W <mark>SRKA</mark> SSKI	M <mark>IW</mark> FT <mark>A</mark> LI	ILSV <mark>N</mark> G	JLM <mark>A</mark> G <mark>YTGLVH</mark>
Bombyx	FRFVLPLLPILLYLAQDAI	VP	W <mark>SRKA</mark> KRW	2 <mark>ly</mark> vv <mark>a</mark> ac	M <mark>VV</mark> G <mark>N</mark> A	L <mark>PA</mark> AYF <mark>G</mark> AVH
Tribolium	FRFLLPLLPIALF <mark>ISS</mark> RFL	SV	W <mark>SRKA</mark> SNVN	M <mark>lw</mark> tv <mark>a</mark> li	LF <mark>V</mark> GNV	G <mark>PSYYLGYVH</mark>
Zebrafish	FRFI <mark>YPVLP</mark> FCMIFCGLSL	ak <mark>l</mark> qa	<mark>W-RKAA</mark>	- <mark></mark> AGA	LLLFNI	CPA <mark>LYT</mark> GLVH
Fugu	FRFI <mark>YPVLPF</mark> CLVFSGI <mark>S</mark> L	AS <mark>LK</mark> S	<mark>w</mark> - <mark>rraa</mark>	<mark>A</mark> F <mark>F</mark>	LLV <mark>S</mark> NI	AA <mark>GLYT</mark> GLIH
Xelaevis	<mark>FRFI<mark>YP</mark>VLP</mark> V <mark>C</mark> MVFCGFSF	SN <mark>LK</mark> R	<mark>w-kkaa</mark>	VG <mark>F</mark>	LVL <mark>S</mark> NI	FPA <mark>LYT</mark> GLIH
Chick	FRFI <mark>YPVLP</mark> FCMFFCGYS <mark>L</mark>	KH <mark>LK</mark> A	<mark>W-KK</mark> S <mark>A</mark>	<mark>ASF</mark>	LLL <mark>S</mark> NI	IPA <mark>LYT</mark> GLIH
Mouse	FRFI <mark>YPVLP</mark> FCMVFCGYS <mark>L</mark>	AH <mark>L</mark> KT	<mark>w-rkaa</mark>	<mark>LSF</mark>	LLL <mark>S</mark> NV	PLAFY <mark>TGL</mark> VH
Rabbit	FRFI <mark>YPVLP</mark> FCMVFCGYS <mark>L</mark>	TH <mark>LK</mark> M	I <mark>W-KK</mark> P <mark>A</mark>	<mark>LSF</mark>	LF <mark>LS</mark> NI	LL <mark>ALYTGLVH</mark>
Human	FRFI <mark>YPVLP</mark> FCMVFCGYS <mark>L</mark>	TH <mark>L</mark> KT	<mark>W-KK</mark> P <mark>A</mark>	<mark>LSF</mark>	lf <mark>ls</mark> ni	FL <mark>ALYT</mark> GLVH

#### Fig. S3. Comparison of the amino acid sequences of PIG-B in various organisms

Amino acid sequences of D4 segment of *Drosophila* and the corresponding segments of *Atta cephalotes prog* (ant), *Apis mellifera* (honeybee), *Anopheles gambiae* (mosquito), *Bombyx mori* (bombyx), *Tribolium castanet* (tribolium), zebrafish, fugu, Xenopus, chick, mouse, rabbit, and human were aligned. Amino acids conserved in most species, insects, and vertebrates are shown in red, yellow, and green, respectively. The corresponding regions exchanged between *Drosophila* and human, ant, mosquito, bombyx, and tribolium are shown in a red box.



# Fig. S4. Localization, activity, rescue efficiency, and expression level of chimeric PIG-Bs containing sequences of *Drosophila* and ant, mosquito, bombyx, or tribolium

- (A) Immunofluorescence analysis of S2 cells expressing myc-tagged chimeric PIG-Bs containing sequences of *Drosophila* and other insects. Cells were stained with anti-myc (green) and anti-lamin (magenta) antibodies. Bar, 10 μm.
- (B) Restoration of surface expression of uPAR on class B mutant CHO cells by transfection of chimeric PIG-Bs. Gray, class B cells; red, parental cell line F21; bold black line, class B cells expressing each chimeric PIG-B.
- (C) Rescue of *PIG-B*<sup>13</sup> lethality by expression of myc-tagged DmPIG-B[NE] and chimeric PIG-Bs under the control of da-Gal4 at 25° C. Values are means  $\pm$  S.D. of data obtained in three independent crosses.
- (D) Immunoblot analysis of myc-tagged DmPIG-B[NE] and chimeric PIG-Bs expressed in adult flies under the control of da-Gal4. Proteins were detected with anti-myc and anti-tubulin (loading control) antibodies.



### Fig. S5. Localization of endogenous DmPIG-O in ML-DmD9 cells

Immunofluorescence analysis of DmPIG-O in ML-DmD9 cells in which GFP (GFP dsRNA) or DmPIG-O (DmPIG-O dsRNA) was knocked down. Cells were stained with an anti-DmPIG-O antibody (green) and DAPI (blue). Arrowheads indicate strong punctate signals of DmPIG-O proximal to the NE. Bar,  $5 \mu m$ .