Supplementary Information

Short-term urea cycle inhibition in rat liver cells induced by polyethylene glycol

Li Xu, [a] Jiapei Yang, [a] Yumin Liu, [c] Leilei Shi, [a] Chenwei Wu, [a] Hua Jin, [a] Xin Jin, [a] Yue Su, [a, **] and Xinyuan Zhu [a, b, *]

[a] School of Chemistry and Chemical Engineering, State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, China

[b] Shanghai Key Laboratory for Molecular Engineering of Chiral Drugs, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, China

[c] Instrumental Analysis Center, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, China

[*] Corresponding author.

E-mail addresses: yuesu@sjtu.edu.cn (Y. Su); xyzhu@sjtu.edu.cn (X. Zhu)

Supplementary Figures

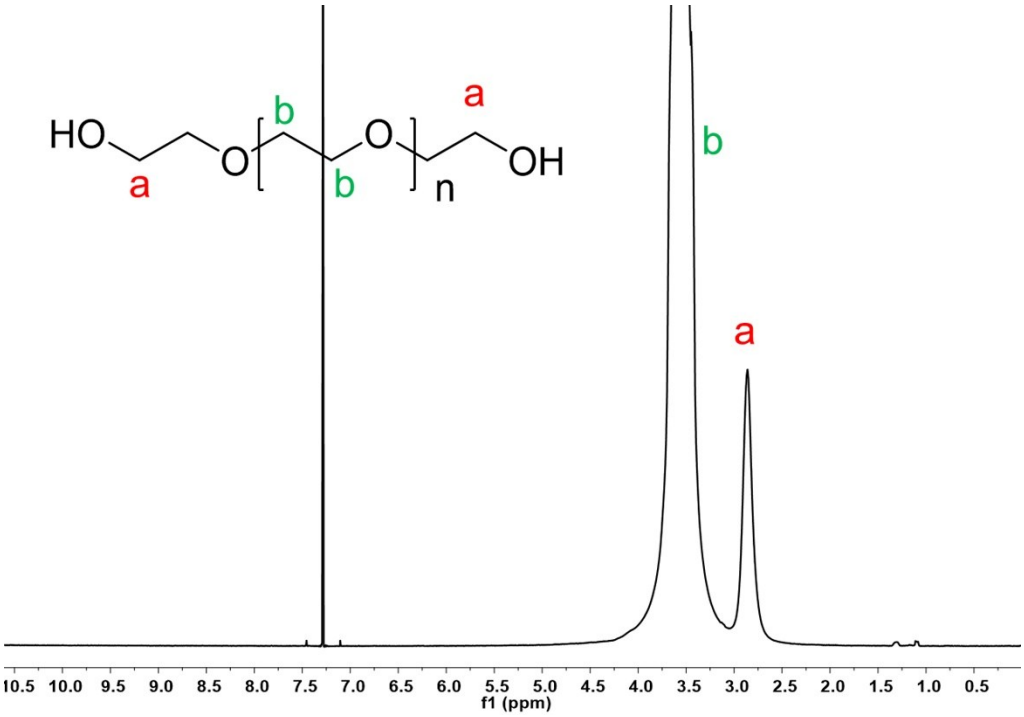

Fig. S1 ^{1}H-NMR spectrum of PEG (600 MHz, $CDCl_3$). ^{1}H-NMR ($CDCl_3$, 600 MHz) d=3.53 (-OCH_2CH_2O-), 2.86 (-CH_2-OH) ppm.

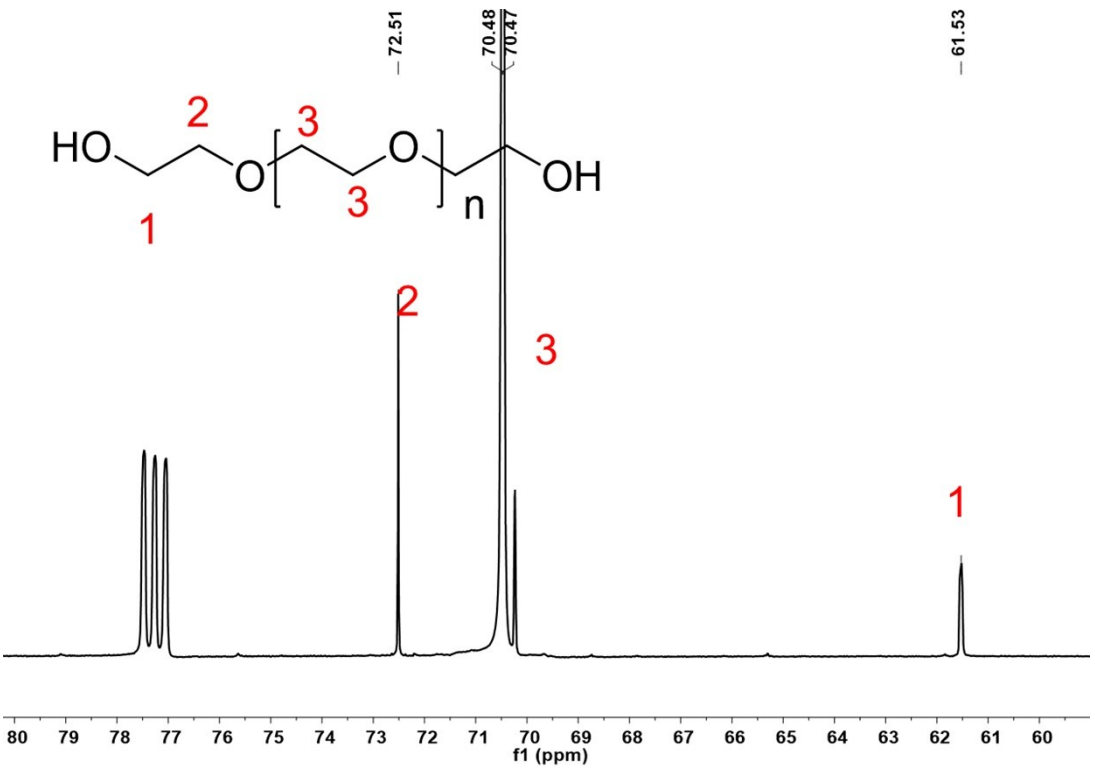

Fig. S2 ^{13}C-NMR spectrum of PEG (150 MHz, $CDCl_3$).^{13}C-NMR ($CDCl_3$, 150 MHz) d = 72.51, 70.48, 70.47, 69.94, 61.53 ppm.

Table S1. The metal ions traces in PEG (2 kD).

Metallic element	PEG nitrate soluble aqueous solution		PEG aqueous solution		Detected limitation (ppm)
	Content (ppm)	%RSD	Content (ppm)	%RSD	
Al	---	---	---	---	0.0087
As	---	---	---	---	0.0114
Ba	---	---	---	---	0.0003
Bi	---	---	---	---	0.0210
Ca	---	---	---	---	0.0072
Cd	---	---	---	---	0.0003
Co	---	---	---	---	0.0021
Cr	---	---	---	---	0.0033
Cu	---	---	---	---	0.0054
Fe	4.39	0.7352	0.098	21.75	0.0030
K	24.49	2.733	2.822	71.41	0.0750
Li	---	---	---	---	0.0030
Mg	0.51	0.6674	0.5013	4.190	0.0003
Mn	0.1155	4.249	0.1623	24.1	0.0003
Mo	---	---	---	---	0.0015
Na	118.45	0.6407	100.1	0.1424	0.0105
Ni	---	---	---	---	0.0036
Pb	---	---	---	---	0.0138
Sr	---	---	---	---	0.0000
Zn	2.397	0.4007	0.2008	79.74	0.0009

The correction factor for samples is 100.

--- indicated an undetected metal ion, the content of which in solution is lower than the detected limitation with %RSD>5.

Table S2. The P values of metabolites shown in the heatmap (Fig. 2a) according to ANOVA analysis with MetaboAnalyst software.

Metabolites	*P* value
Urea	1.74E-07
GABA	5.3E-08
Glucose-1-phosphate	1.32E-07
Putrescine	8.58E-16
alpha-Aminoadipic acid	6.19E-07
Methyl-beta-D-galactopyranoside	7.06E-08
Myo-inositol	8.93E-15
D-(glycerol 1-phosphate)	1.86E-11
beta-Glycerophosphoric acid	1.9E-09
Oxoproline	4.66E-10
Palmitoleic acid	3.09E-08
Oleic acid	2E-07
21-hydroxypregnenolone	5.02E-08
Methyl hexadecanoate	3.11E-13
Myo-inositol	4.48E-12

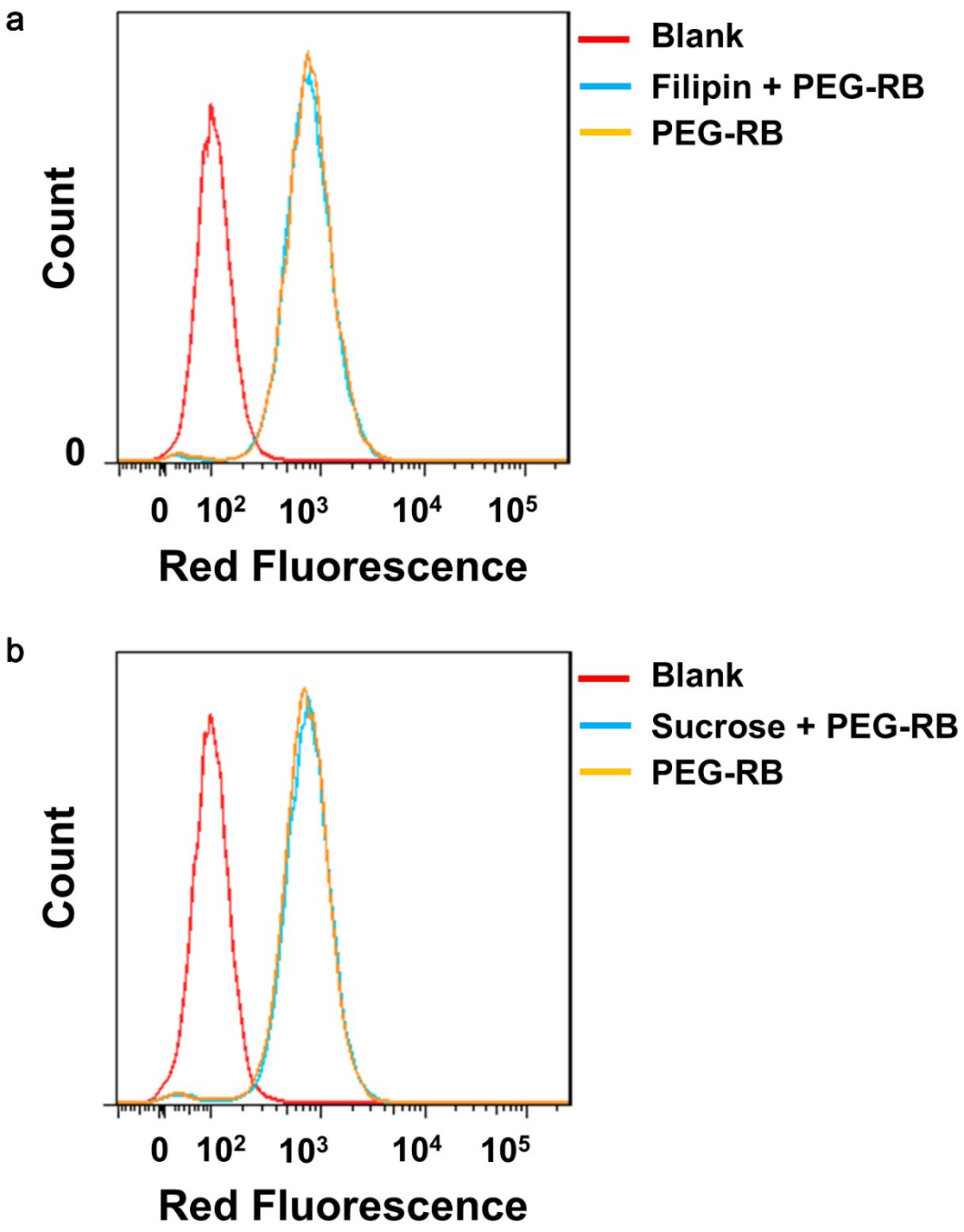

Fig. S3 Inhibition of clathrin-and caveolar-mediated pathways does not affect the internalization of PEG-RB (10 μM). The caveolar-mediated pathway (a) was Inhibited by filipin (7.5 μM) and the clathrin-mediated pathway (b) was Inhibited by sucrose (450 mM).

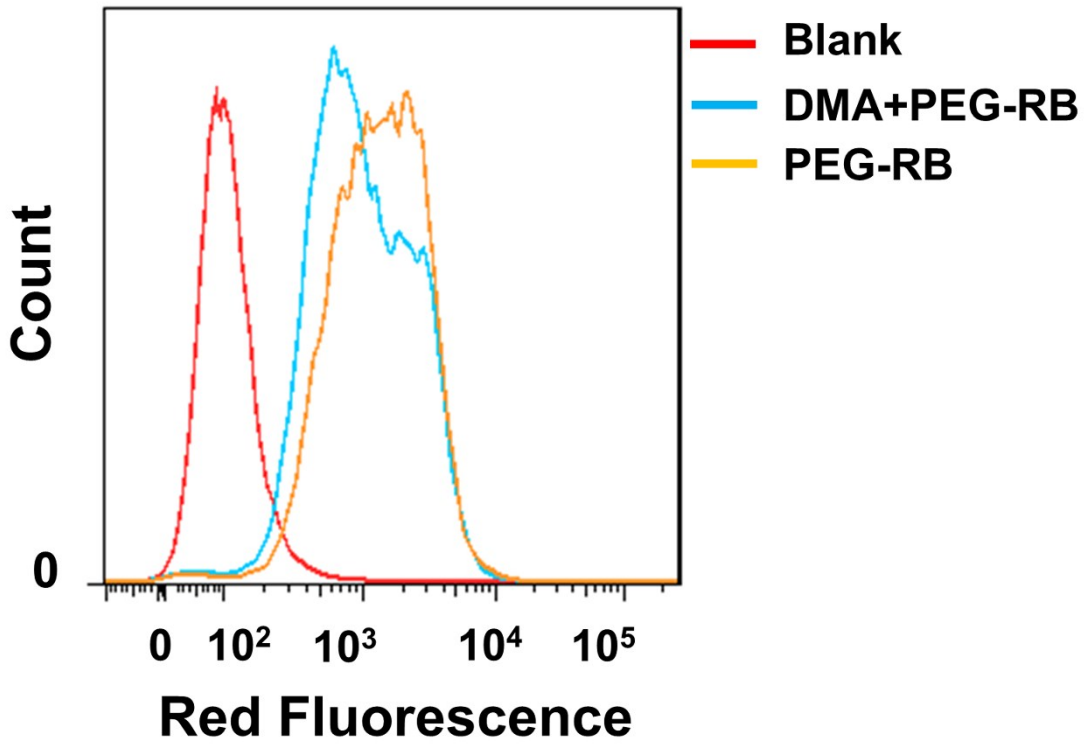

Fig. S4 Inhibition of macropinocytosis pathways affects the internalization of PEG-RB (10 μM). The macropinocytosis was inhibited by 5-(N,N-dimethyl) amiloride hydrochloride (DMA, 400 μM).

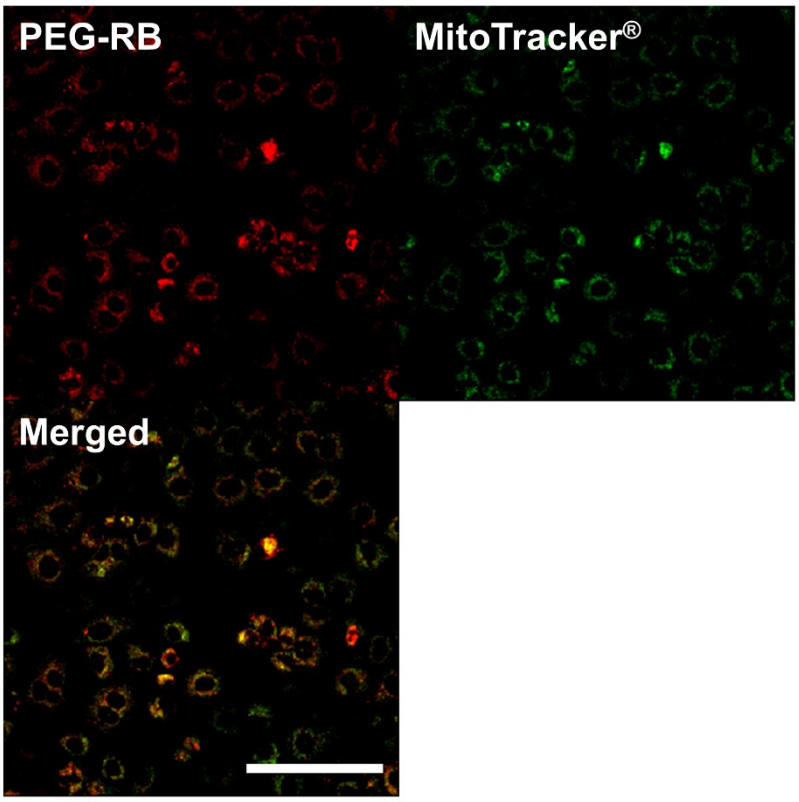

Fig. S5 The representive confocal images of the colocalization of PEG-RB and mitochondria. The mitochondria was labeled by MitoTracker® probes (100 nM). Scale bar: 100 μm.

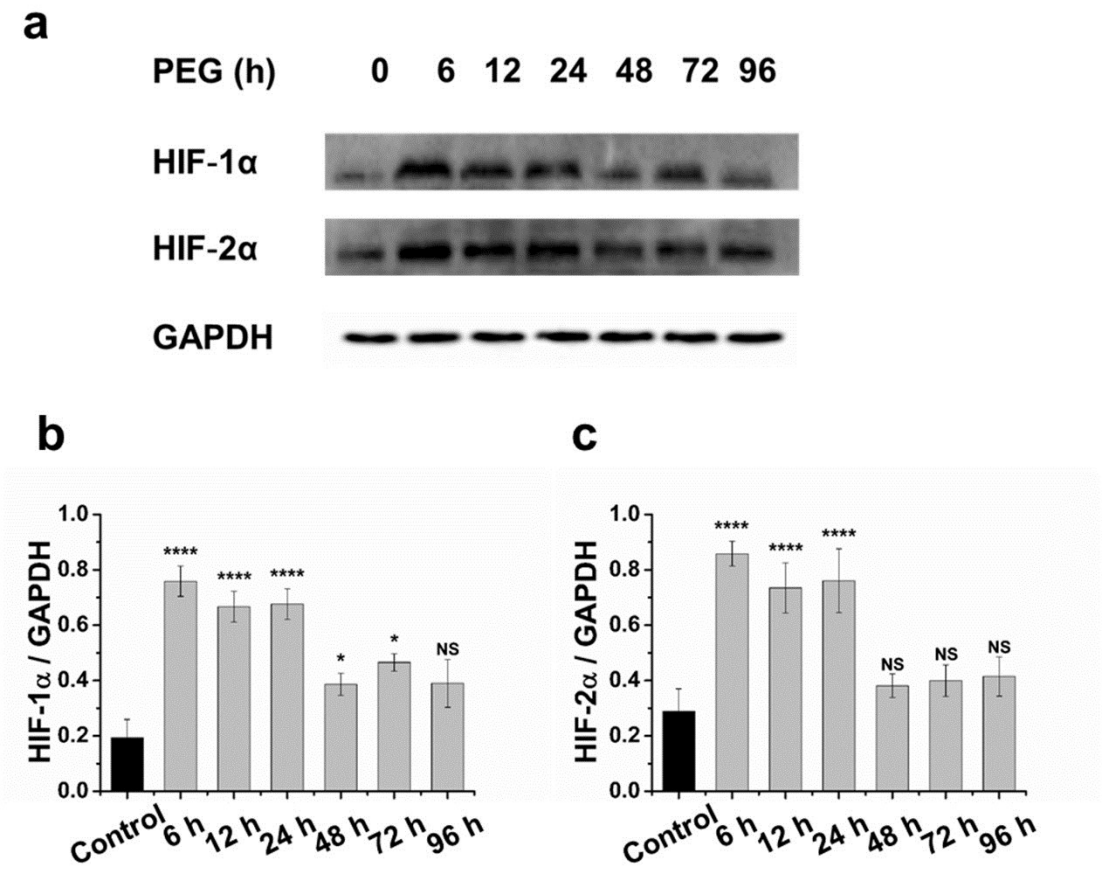

Fig. S6 (a) The intracellular HIF-1α and HIF-2α expressions in control and test groups treated with PEG (2 kD, 20 mM) for different time (6, 12, 24, 48, 72 and 96 h) according to western blot analysis. (b and c) The relative HIFs (HIF-1α/GAPDH and HIF-2α/GAPDH) expressions according to (a). $^{*}P<0.05$, $^{****}P<0.0001$, NS, not significant.

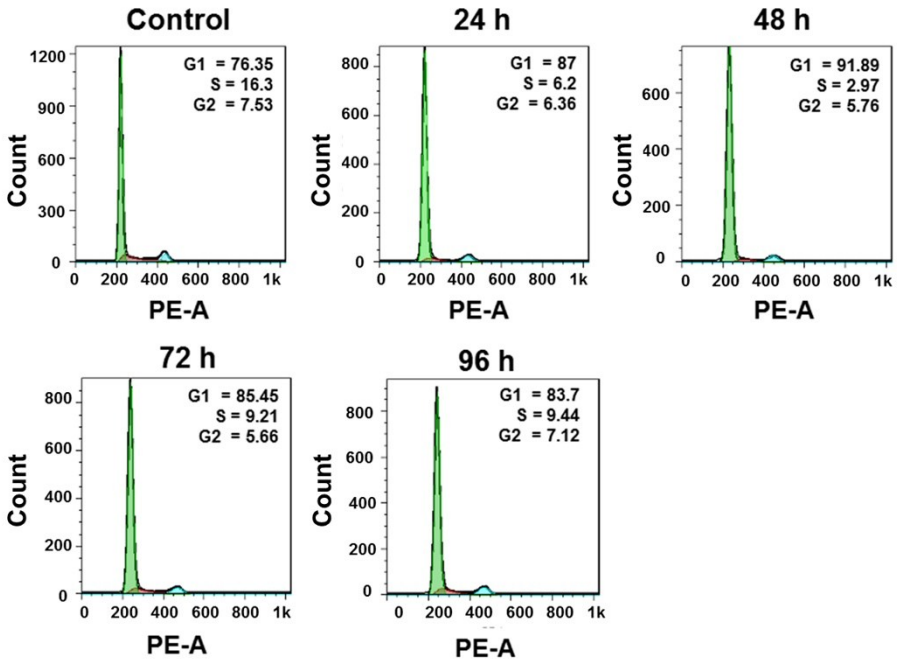

Fig. S7 PEG leads to a reduced cell growth. The cell cycle analysis of BRL-3A cells co-cultured with PEG (2 kD, 20 mM) for different time (24 h, 48 h, 72 h, 96 h).

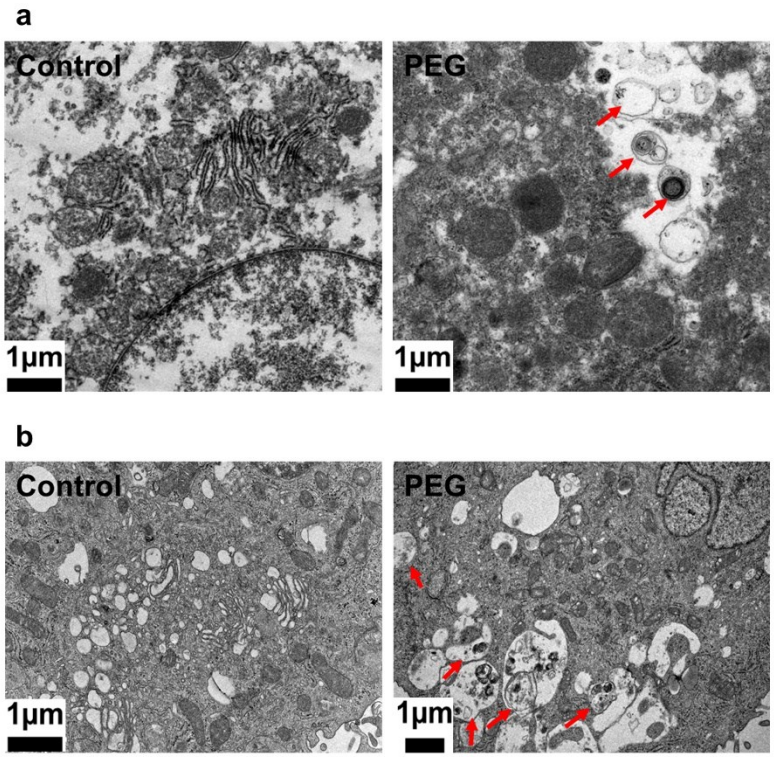

Fig. S8 PEG induces autophagosomes in liver both *in vitro* and *in vivo*. **(**a**)** TEM images of liver in rats at 72 h in control and PEG (2 kD, 8 mg/g bw) treated groups. **(**b**)** TEM images of liver cells (BRL-3A cells) at 24 h in control and PEG (2 kD, 20 mM) treated groups. The arrow indicates autophagosomes.

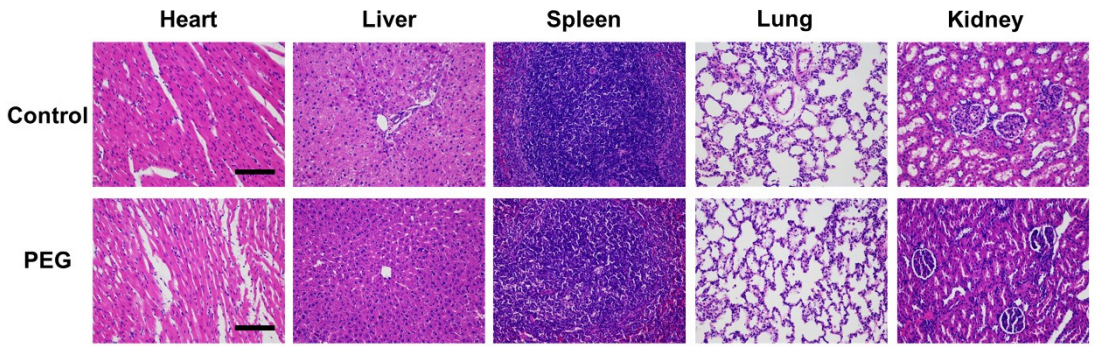

Fig. S9 PEG has no effects on the tissues. Representative images of hematoxylin-eosin staining slices of heart, liver, spleen, lung and kidney in rats at 72 h after administration of PEG (2 kD, 8 mg/g bw). Scale bar: 100 μm.