Supplemental Figure 1A, B.

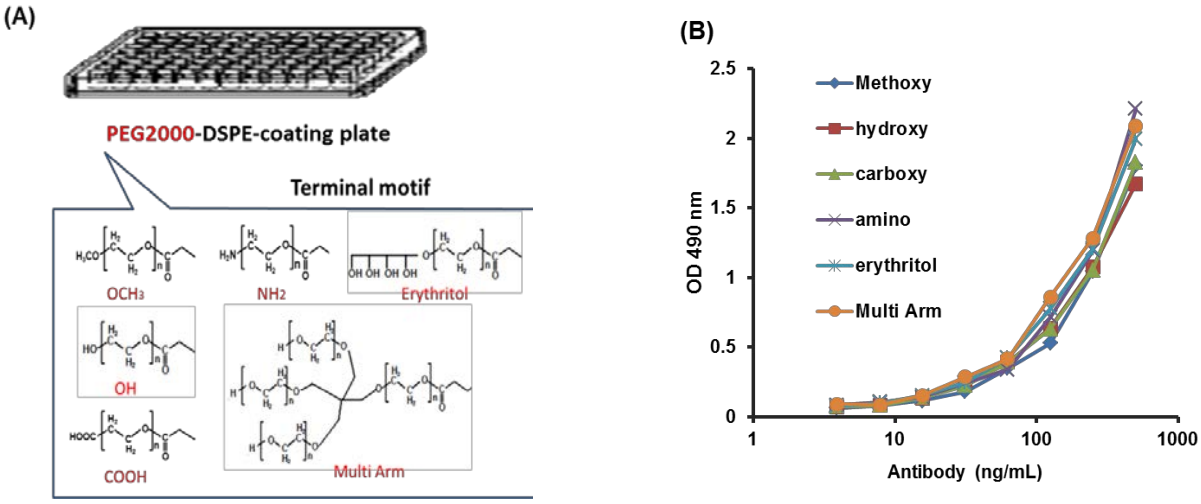

Fig. S1, Monoclonal anti-PEG IgG (HIK-G11) binding assay against various terminal-motifs of PEG-DSPE

A binding assay of HIK-G11 (mouse $IgG_1\kappa$) was conducted using 96-well plates coated with various types of PEG-DSPE via ELISA. Blocking buffer (50 mM Tris, 0.14 M NaCl, 1% BSA) was added and incubated for 1 hr. Then, solutions of HIK-G11 with different concentrations were applied and incubated for 1 hr at room temperature. The secondary antibody (HRP-conjugated anti-mouse IgG, whole molecule) was diluted 1/2,000. Terminal-motifs of PEG-DSPE used in our experiment are illustrated in Fig. S1A. As shown in Fig. S1B, HIK-G11 bound all terminal-motifs of PEG-DSPE that were tested, which suggested that the antibody recognized repeated oxyethylene units of PEG. No detectable binding against OVA was confirmed (data not shown).

Supplemental Figure 2A, B.

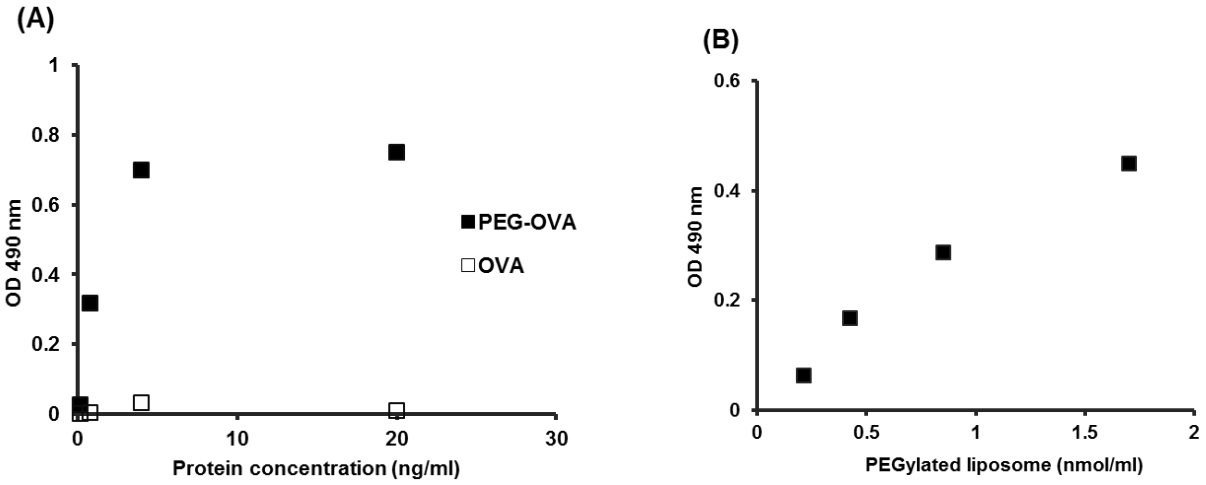

Fig. S2, Detection of PEGylated products by sandwich ELISA

To quantitate PEGylated products, we developed a sandwich ELISA with our monoclonal anti-PEG IgG (HIK-G11). The conditions of the sandwich ELISA are described in the Experimental Section. Fig. S2A shows that PEG-OVA detection ranged between 0.8-5 ng/ml in this system. In addition, this system may be able to measure concentration of PEGylated liposome (Fig. S2B).

Supplemental Figure 3A, B.

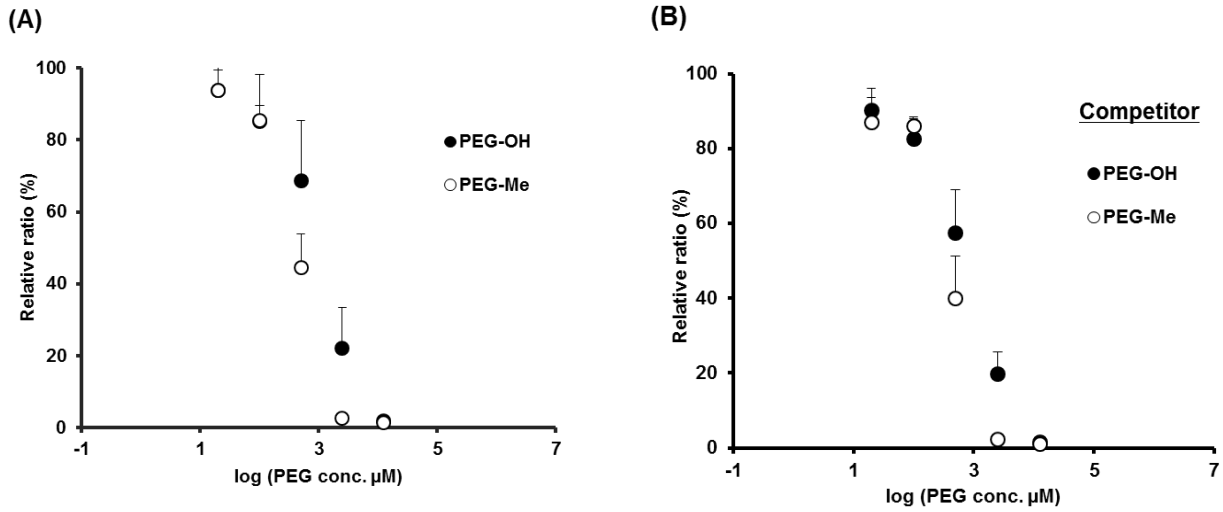

Fig. S3. Determination of whether the anti-PEG IgM induced by PEG-OVA and PEGylated liposome is "terminal methoxy-specific" or "backbone-specific"

To determine the selectivity of anti-PEG IgM, competitive ELISA was carried out using similar methods to the ELISA demonstrated in the Experimental Section. The binding of anti-PEG IgM in 50 μl of serum diluted 100-fold to $mPEG_{2000}$-DSPE on the plate was determined in the presence of various amounts of competitors (PEG_{2000}-OH or PEG_{2000}-methoxy). As shown in Fig. S3, the anti-PEG IgMs induced by (A) PEG-OVA and (B) PEGylated liposome showed similar selectivity against PEG_{2000}-OH or PEG_{2000}-methoxy, although they showed a slightly strong affinity to PEG_{2000}-methoxy. This result suggests that the anti-PEG IgMs in the present study mainly bound to the repeating oxyethylene units (backbone) of PEG.