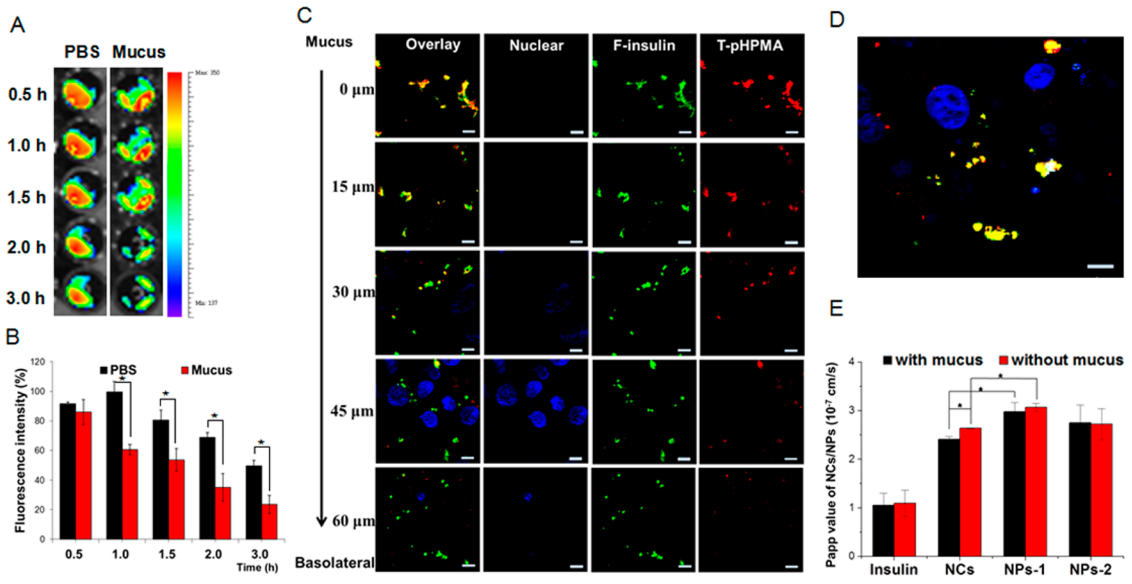

Figure 3. (A) FRET intensity of particles prepared with F-insulin and T-pHPMA after incubation in PBS or mucus for different times. The fluorescent intensity of TRITC represented the structural integrity of particles. (B) Quantitative analysis of FRET intensity for particles in PBS or mucus. Data was presented as the percentage of the intensity before incubation. Data are means ± SD ($n = 3$), $^{\star}p < 0.05$. (C) CLSM images of the distribution of F-insulin and T-pHPMA in NPs on E12 cell monolayer from apical to basolateral side. Red, T-pHPMA; green, F-insulin; yellow, colocalization of F-insulin and T-pHPMA. Scale bars, 5 μm. (D) CLSM images of E12 cells after incubation with NPs prepared with F-insulin and T-penetratin. Red, T-penetratin; green, F-insulin; yellow, colocalization of F-insulin and T-penetratin. Scale bars, 5 μm. (E) Papp value of different samples across the E12 monolayer in the transepithelial transport study. Data are means ± SD ($n = 3$), $^{\star}p < 0.05$.

has no significant influence on the transport of NPs-1 and NPs-2, while it reduced the transport of NCs. Besides, it was observed that the transepithelial electrical resistance (TEER) values of the cell monolayers were unchanged over time with the incubation of tested samples (Supporting Information, Figure S10), which indicated that the cell monolayer was intact and the translocation was only through a transcellular pathway.

Intracellular progression is a vital part of transcellular transport process. To investigate the intracellular trafficking of NCs and NPs, we first studied the localization of fluorescent labeled insulin in different organelles. The organelles, including endoplasmic reticulum (ER), Golgi apparatus, mitochondria and lysosome were stained using specific probes (red fluorescence). As shown in Figure 4A, colocalization of F-insulin with all of the four organelles was observed for both NCs and NPs. To better understand of the participation of the organelles in the particle progression, we then investigated the involvement of different organelles in the exocytosis of the particles. Previous reports demonstrated that ER and Golgi apparatus are vital components for secretory ER/Golgi pathway and endocytic recycling pathway.[33] Moreover, lysosomes also act as an important regulator for the exocytosis of internalized particles *via* fusion of the lysosomal membrane with plasma membrane.[34] Exocytosis of fluorescent-labeled particles on E12 cells was observed of all tested formulations (NCs, NPs-1 and NPs-2) as demonstrated in a chase-pulse study (Supporting Information Figure S11). Furthermore, the exocytosis followed a time and energy dependent manner (Supporting Information Figures S12 and S13). We investigated the inhibition of exocytosis using specific progression inhibitors, including brefeldin A (ER/Golgi secretory pathway inhibitor), monensin (a Golgi/recycling endosome exocytosis pathway inhibitor), LY294002 and nocodazole (lysosomal exocytosis inhibitors). Incubation of the inhibitors with cells exhibited specific influence on the morphology of the corresponding organelles (Figure 4B). Both brefeldin A and monensin resulted in a remarkable inhibition of exocytosis of NCs or NPs (Figure 4C), suggesting the involvement of ER and Golgi apparatus in their intracellular trafficking. LY294002 and nocodazole also resulted in reduction their exocytosis (Figure 4D). These results indicated that ER, Golgi apparatus and lysosome were all involved in the intracellular trafficking of NCs and NPs. The pHPMA coating of the NPs did not affect the progression of the encapsulated drug relative to NCs.

***In Vivo* Hypoglycemic Effect and Pharmacokinetics.** Finally, we evaluated the hypoglycemic effect and pharmacokinetics following oral administration of the NPs on diabetic rats. As shown in Figure 5A, similar to the saline, oral administration of free insulin solution failed to reduce the blood glucose level, while the administration of NPs-1 generated a remarkable hypoglycemic response with maximal 50% of blood glucose level reduction. The blood glucose level of the NPs-1 group was remained under 200 mg/dL for ~3 h, which is the