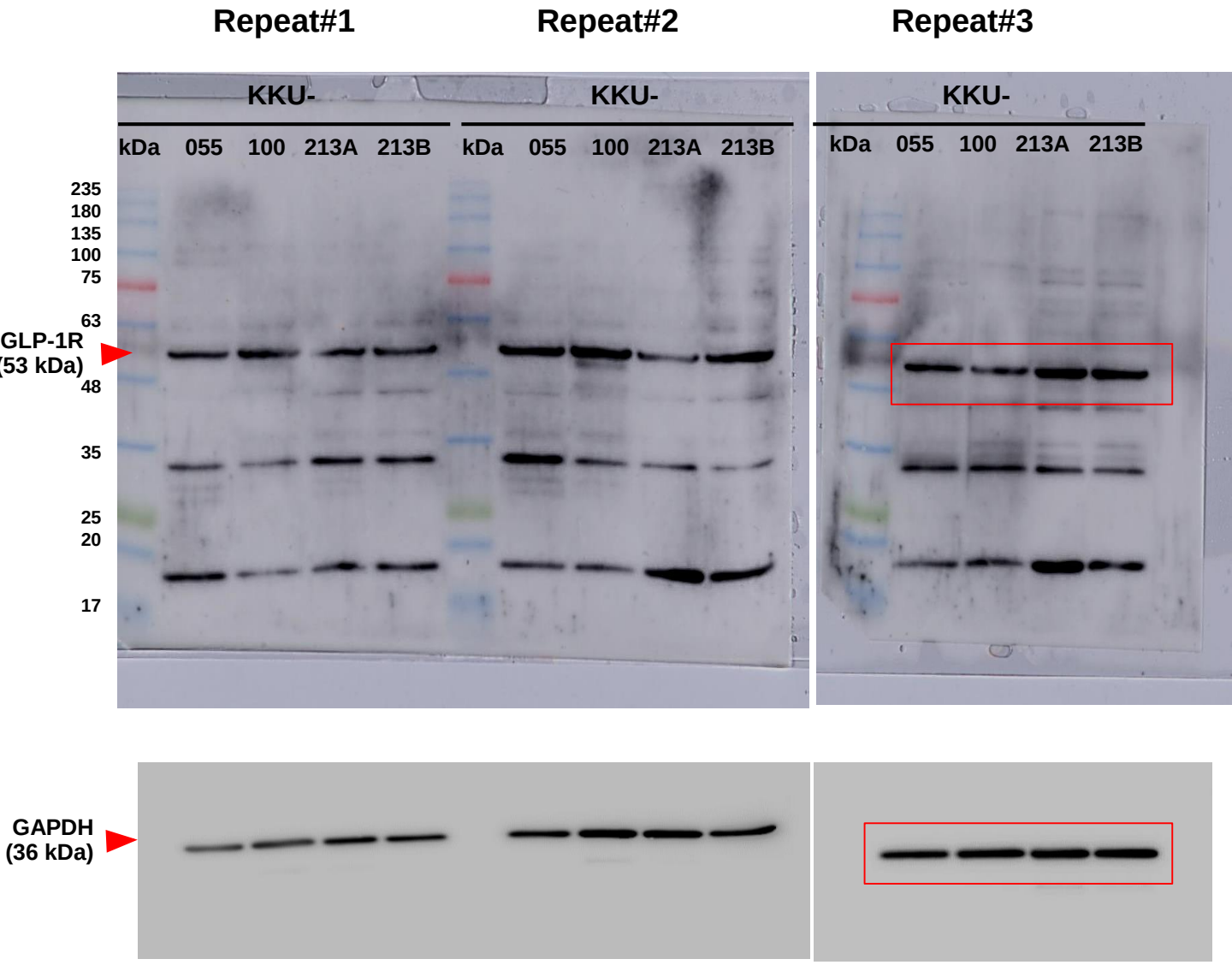
Supplementary data

Liraglutide exhibits potential anti-tumor effects on the progression of intrahepatic cholangiocarcinoma, in vitro and in vivo

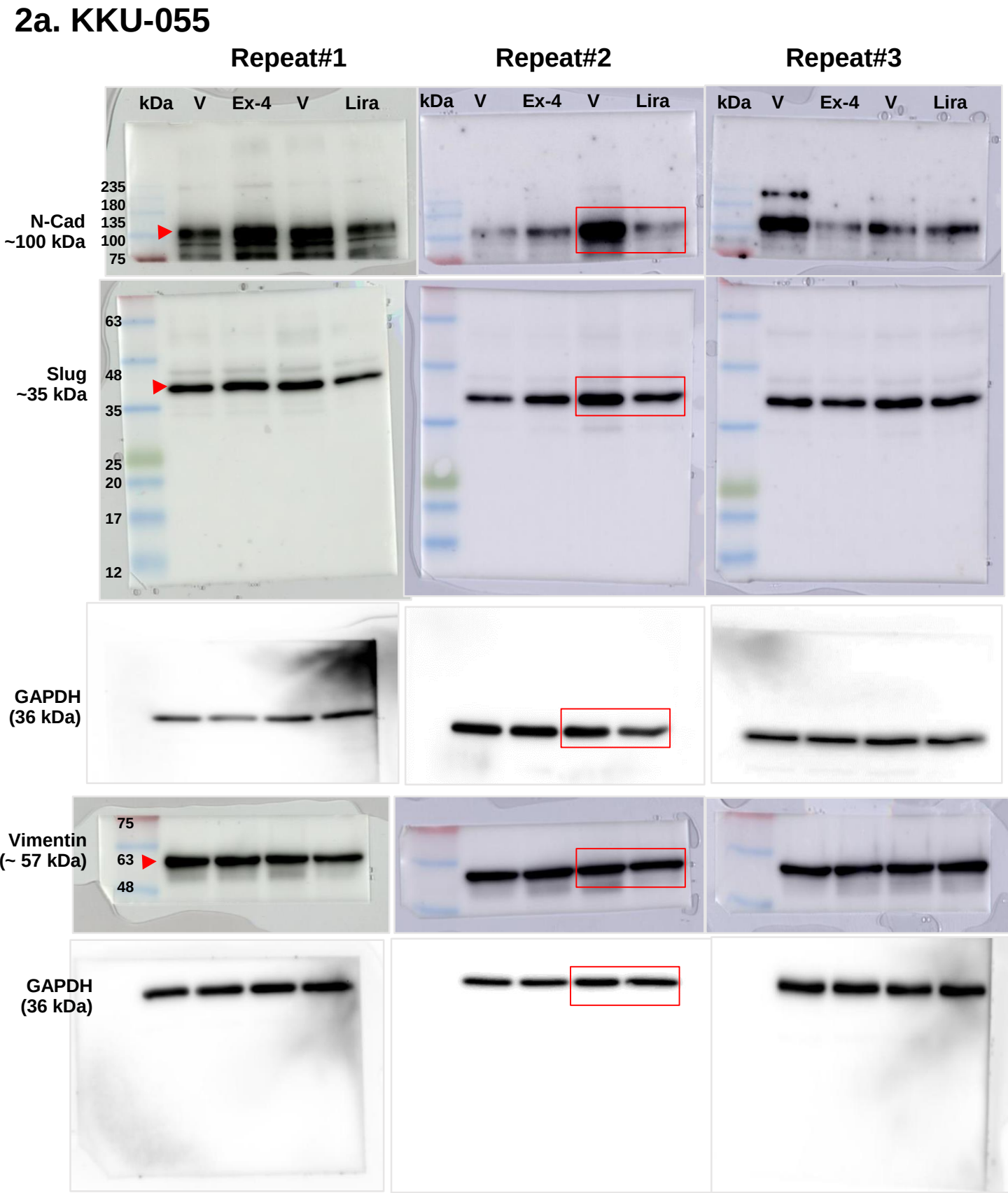
Ronnakrit Trakoonsenathong[a, b], Waritta Kunprom[c], Chaiwat Aphivatanasiri[c, e], Padcharee Yueangchantuek[b], Paslada Pimkeeree[b, d], Supannika Sorin[b, e], Kullanat Khawkhiaw[b, e], Ching-Feng Chiu[f], Seiji Okada[g], Sopit Wongkham[b, e], Charupong Saengboonmee[a, b, e]*

[a]Cho Kalaphruek Excellent Research Program for Medical Students, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
[b]Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
[c]Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
[d]Faculty of Medical Sciences, Naresuan University, Phitsanulok, Thailand
[e]Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand
[f]Graduate Institute of Metabolism and Obesity Sciences, Taipei Medical University, Taipei, Taiwan
[g]Division of Hematopoiesis, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Japan

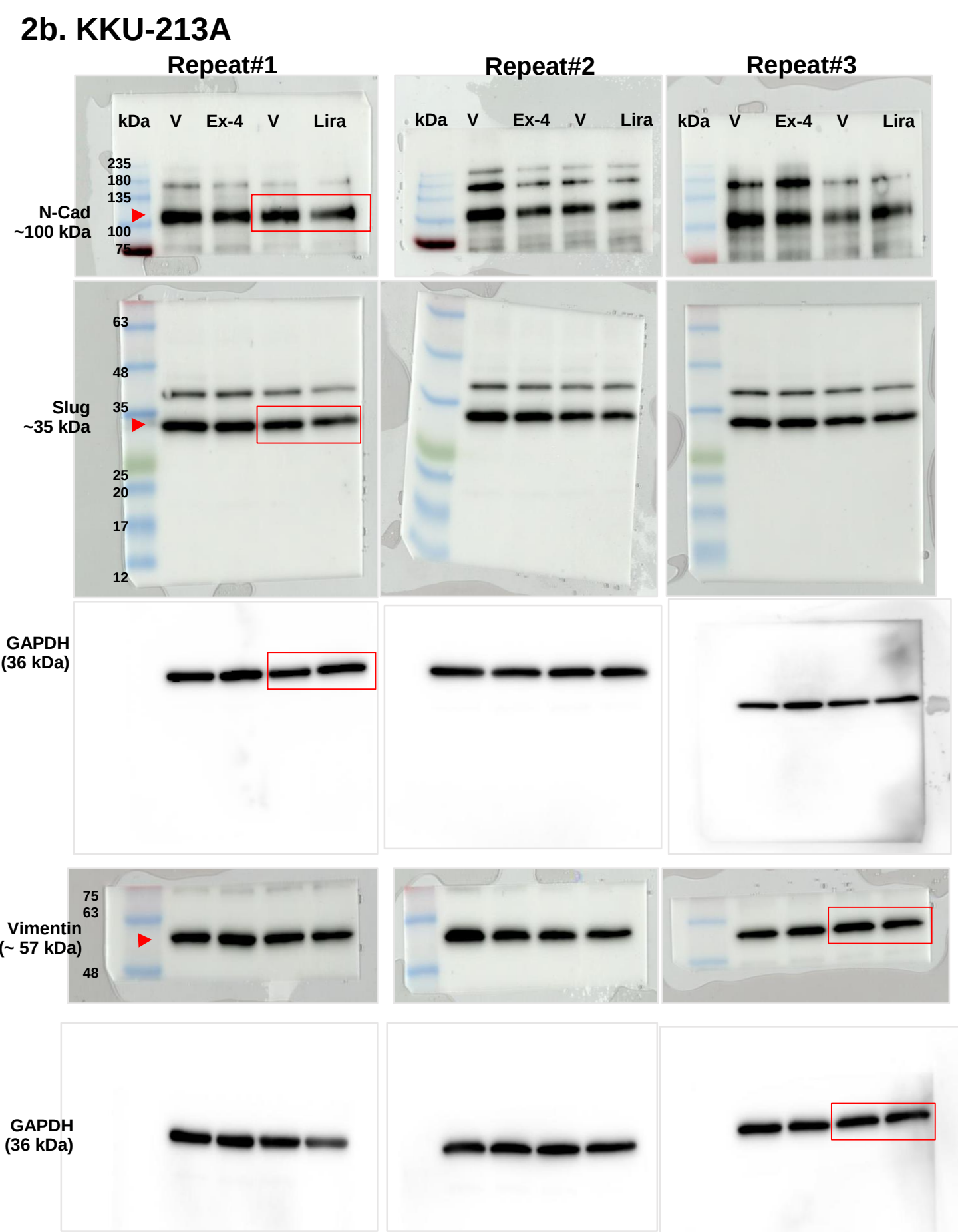
Send correspondence to:
Asst. Prof. Charupong Saengboonmee, M.D., Ph.D.
Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand, Tel & Fax: +66-4336-3265, Email: charusa@kku.ac.th



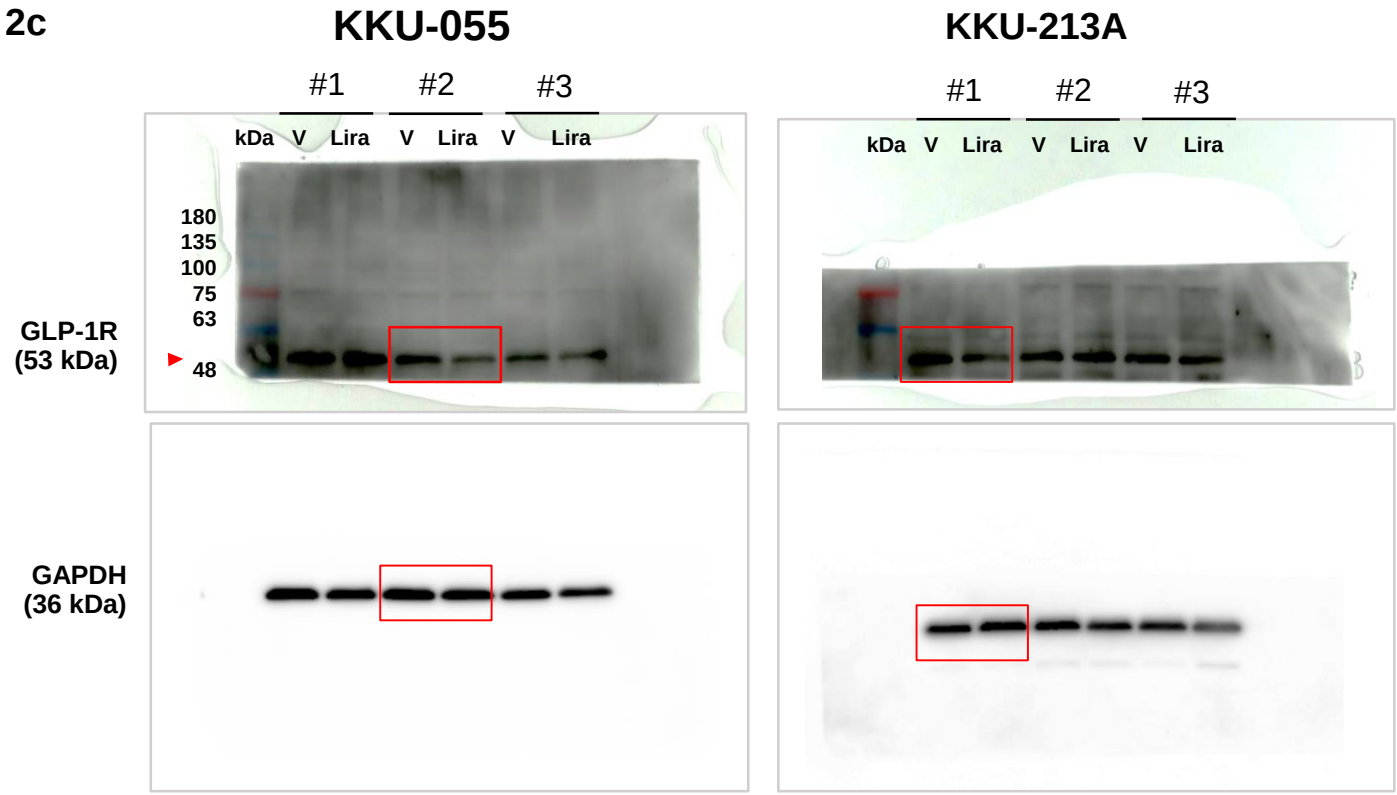
Supplementary Figure S1. Glucagon-like peptide 1 receptor expression in 4 cholangiocarcinoma cell lines.



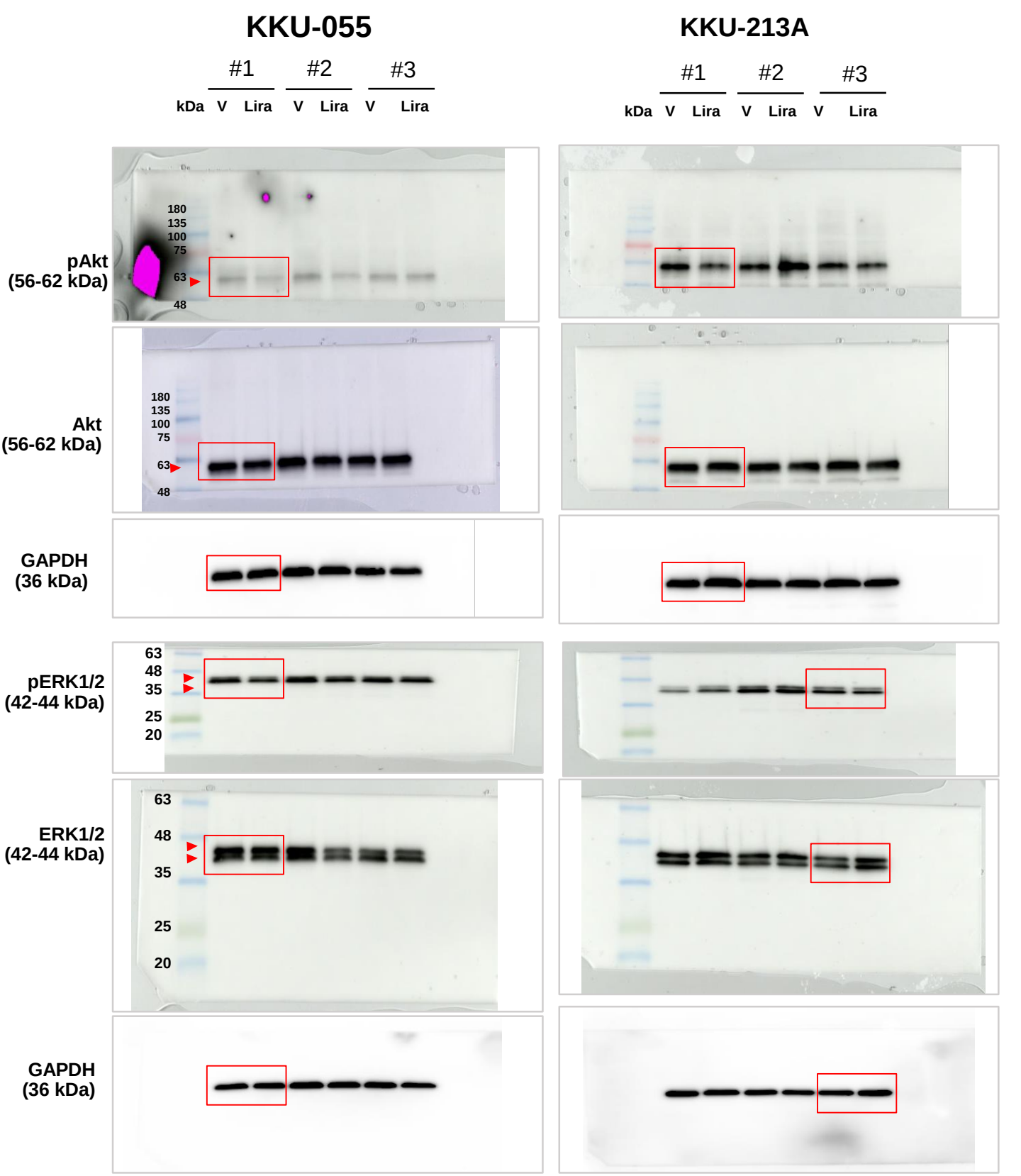
Supplementary Figure S2. Effects of glucagon-like peptide 1 receptor agonists (GLP-1Ra) on the expressions of GLP1R and epithelial-mesenchymal (EMT) markers in cholangiocarcinoma cell lines. Liraglutide suppresses expressions of EMT markers in (a) KKU-055, and (b) KKU-213A. (c) Liraglutide suppresses expression of GLP1R. In both CCA cell lines. Ex-4: Exendin-4, Lira: Liraglutide



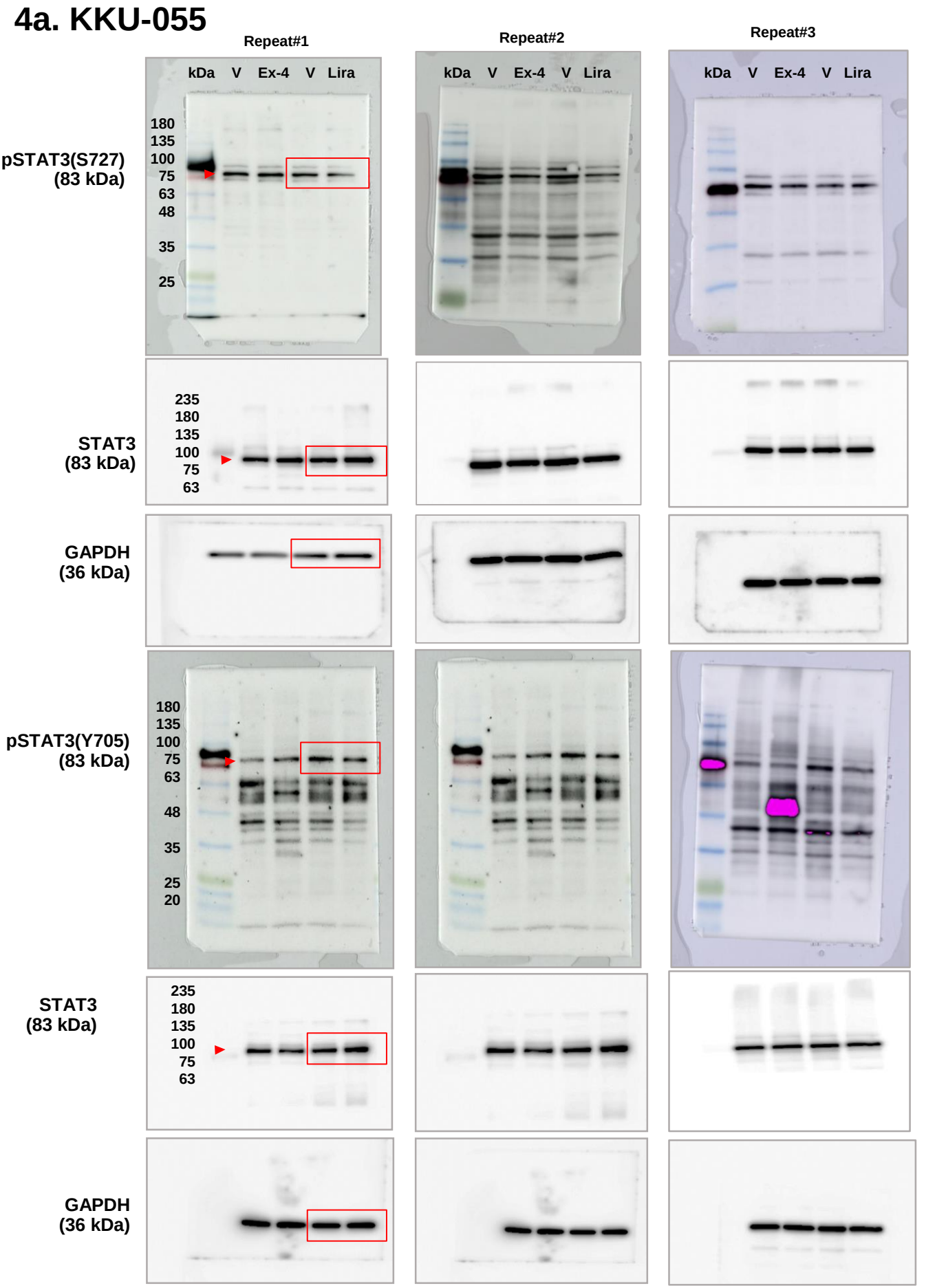
Supplementary Figure S2. Effects of glucagon-like peptide 1 receptor agonists (GLP-1Ra) on the expressions of GLP1R and epithelial-mesenchymal (EMT) markers in cholangiocarcinoma cell lines. Liraglutide suppresses expressions of EMT markers in (a) KKU-055, and (b) KKU-213A. (c) Liraglutide suppresses expression of GLP1R. In both CCA cell lines. Ex-4: Exendin-4, Lira: Liraglutide



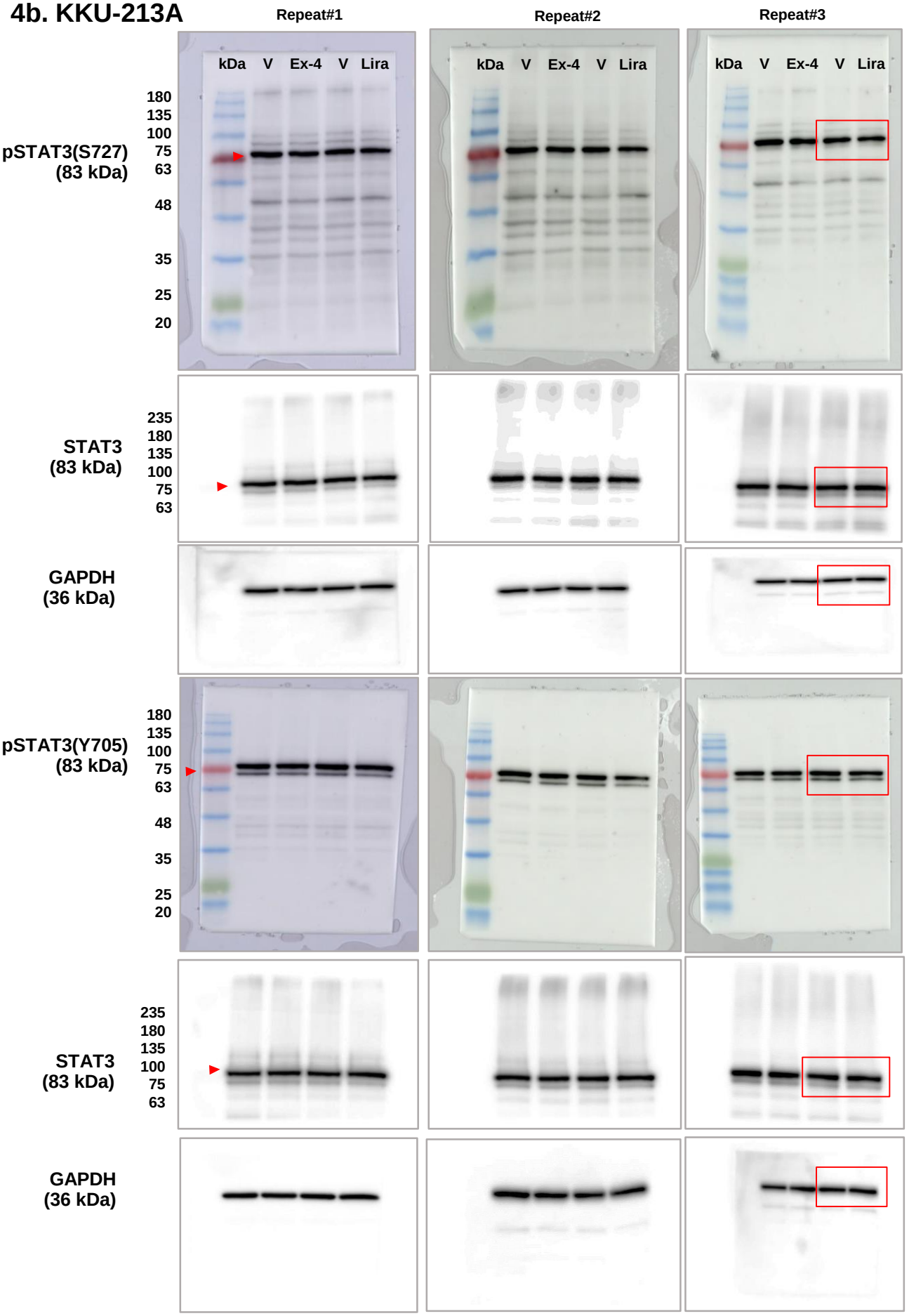
Supplementary Figure S2. Effects of glucagon-like peptide 1 receptor agonists (GLP-1Ra) on the expressions of GLP1R and epithelial-mesenchymal (EMT) markers in cholangiocarcinoma cell lines. Liraglutide suppresses expressions of EMT markers in (a) KKU-055, and (b) KKU-213A. (c) Liraglutide suppresses expression of GLP1R. In both CCA cell lines. Ex-4: Exendin-4, Lira: Liraglutide



Supplementary Figure S3. Liraglutide suppresses the phosphorylation of Akt. Akt phosphorylation was suppressed after cholangiocarcinoma cells were treated with liraglutide.

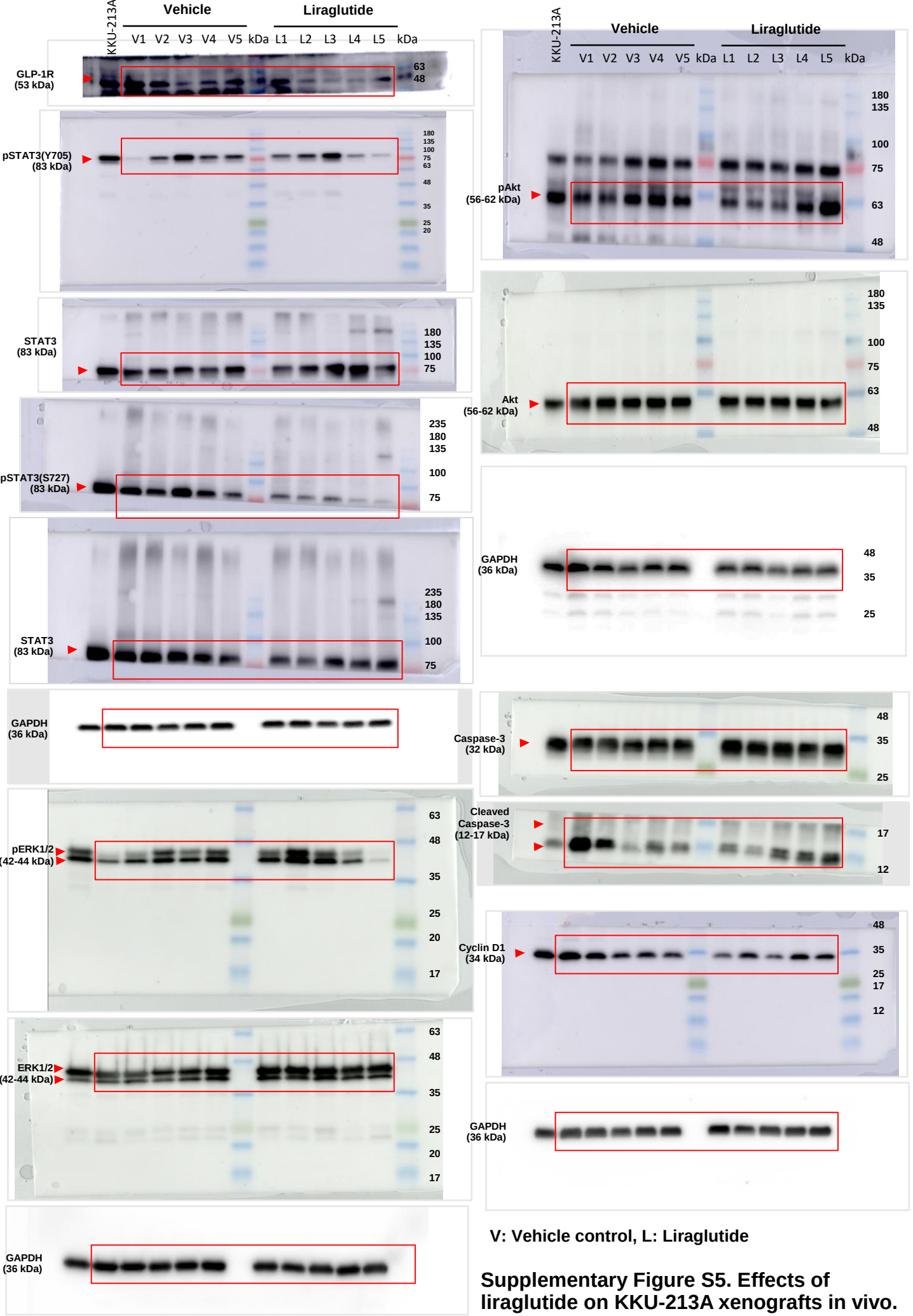


Supplementary Figure S4. Liraglutide suppresses the phosphorylation of STAT3. STAT3 phosphorylation was suppressed after cholangiocarcinoma cells were treated with liraglutide at both Y705 and S727 in both (a) KKU-055 and (b) KKU-213A.



V: Vehicle control, Ex-4: Exendin-4, Lira: Liraglutide

Supplementary Figure S4. Liraglutide suppresses the phosphorylation of STAT3. STAT3 phosphorylation was suppressed after cholangiocarcinoma cells were treated with liraglutide at both Y705 and S727 in both (a) KKU-055 and (b) KKU-213A.



V: Vehicle control, L: Liraglutide

Supplementary Figure S5. Effects of liraglutide on KKU-213A xenografts in vivo.

Supplemental Table S1. Patients characteristics

	Patients with CCA
Age (n=30), [years, median and range]	56 [37-79]
Preoperative FBG (n=25), [mg./dL, mean ± SD, range]	145.48 ± 69.35 [72.00 – 346.00]
Diabetic status (n=25), [%]	
Diabetic-range preoperative FBG	13 [52]
Normal preoperative FBG	12 [48]
Sex (n=30), [%]	
Male	19 [63.30]
Female	11 [36.70]
Histological Grading (n=24), [%]	
Well differentiated	16 [66.70]
Moderately differentiated	6 [25.00]
Poorly differentiated	2 [8.30]
Vascular invasion (n=29), [%]	
Positive	23 [79.30]
Negative	6 [20.70]
Tumor longest diameter (n=28), [cm, mean ± SD, range]	6.64 ± 4.07 [1.30-18.00]
Regional lymph node metastasis (n=28), [n, %]	
Positive	10 [35.70]
Negative	18 [64.30]
Staging of CCA (n = 26) [n, %]	
I-III	17 [65.40]
IV	9 [34.6]
Tumor markers	
CA19-9 (n=12), U/ml, Median [IQR, range]	45.45 [145.11, 3.49 - 735]
CEA (n=12), ng/ml, Median [IQR, range]	4.35 [6.96, 1.33-402.00]