Supplemental Material to:

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dCK expression correlates with 5-fluorouracil efficacy and HuR cytoplasmic expression in pancreatic cancer: A dual-institutional follow up with the RTOG 9704 trial

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Clinical Trial RTOG 97-04

Protocol

An intergroup trial was conducted by the following US National Cancer Institute–sponsored cooperative groups: the Radiation Therapy Oncology Group (RTOG), the Eastern Cooperative Oncology Group, and the Southwest Oncology Group, inclusive of Canadian affiliates. The RTOG served as the lead group with the trial designation RTOG 97-04.

The eligibility criteria included histologically confirmed adenocarcinoma of the pancreas and gross total tumor resection, confirmed by central review of operative and pathology reports. In addition, postoperative computed tomographic (CT) imaging was required within 3 weeks of randomization to exclude patients who had evidence of persistent or recurrent local disease or developed metastatic disease prior to therapy. Surgical resection margins were categorized as negative, microscopically positive, or unknown (defined as those having no comment regarding margins in the pathology report).

Eligibility requirements also included stages T1 to T4, N0 to N1, and M0 according to the 1997 staging criteria of the American Joint Commission on Cancer (see **Box 1** below). If there were no identifiable lymph nodes within the resection specimen, the patient was ineligible. Patients were required to have a Karnofsky performance status of 60 or higher; adequate hematologic, renal, and hepatic function as defined by the following: a white blood cell count of $3 \times 10^3/\mu L$ or higher, a platelet count of $100 \times 10^3/\mu L$ or higher, serum bilirubin and creatinine less than 1.5 × the upper limit of institutional normal, a serum aspartate aminotransferase concentration 5 × the upper limit of institutional normal, and a documented caloric intake of more than 1500 kcal/d. Patients with any prior radiotherapy to any site or chemotherapy were ineligible for this study, as were patients with any prior malignancy other than nonmelanoma of the skin or in situ of the cervix. The serum tumor marker CA 19-9 was submitted for central testing and review. Protocol therapy was required to begin 3 to 8 weeks after resection and within 5 days of randomization. All patients required written and informed consent according to institutional and federal guidelines. All institutions were required to have current institutional review board approval on file with their respective group prior to registration of any patients. The trial was routinely monitored for excessive toxicity by the RTOG Data Monitoring Committee, which functions independently of the RTOG.

Box 1. 1997 Staging Criteria of the American Joint Commission on Cancer

Primary tumor
- T1: Tumor limited to the pancreas and 2 cm or less in greatest dimension
- T2: Tumor limited to the pancreas and more than 2 cm in greatest dimension
- T3: Tumor extends directly into any of the following: duodenum, bile duct, peripancreatic tissues
- T4: Tumor extends directly into any one of the following: stomach, spleen, colon, adjacent large vessels

Regional lymph nodes
- N0: No regional lymph node metastasis
- N1: Regional lymph node metastasis

Distant metastasis
- M0: No distant metastasis
- M1: Distant metastasis

Treatment plan

After undergoing tumor resection, patients were randomly assigned to either fluorouracil (group 1) or gemcitabine (group 2). Randomization was performed 3 to 8 weeks after surgery by a dynamic balancing procedure, which included stratification according to tumor diameter (<3 cm vs ≥3 cm), nodal status (negative vs positive), and surgical margins (negative vs positive vs unknown). Chemotherapy prior to chemoradiation therapy in group 1 consisted of a continuous infusion of 250 mg/m^2 of fluorouracil per day for 3 weeks. Chemotherapy prior to chemoradiation therapy in group 2 consisted of a 30-minute infusion of 1000 mg/m^2 of gemcitabine once weekly for 3 weeks. Between 1 and 2 weeks after completion of chemotherapy, chemoradiation was initiated and was the same for both groups (50.4 Gy with a continuous infusion of 250 mg/m^2 of fluorouracil daily throughout radiation therapy).

Another phase of chemotherapy was initiated 3 to 5 weeks after completion of chemoradiation therapy. Group 1 received 3 months of a continuous infusion of fluorouracil daily [(4 weeks on plus 2 weeks off) × 2]. Group 2 received 3 months of gemcitabine [(3 weeks on plus 1 week off) × 3]. Radiation therapy was delivered in 28 fractions (5 days per week) to the tumor bed and regional nodes. The tumor bed was defined by preoperative CT imaging. Local pancreatic, celiac, mesenteric, periaortic, pancreatic, duodenal, and hepatic portal lymph nodes were included in the radiation therapy fields. After an initial dose of 45 Gy, the final dose of 5.4 Gy was limited to the tumor bed as defined by the preoperative tumor volume. At least 4-MV photons and a minimum 3 to 4 field approach was used. Doses were limited to less than 60% of hepatic volume receiving more than 30 Gy. At least two-thirds of one functioning kidney was spared from radiation therapy fields and the spinal cord was limited to less than 45 Gy. Prospective quality assurance of radiation therapy was required for all. This was inclusive of submission of a preoperative abdominal CT scan and radiation therapy fields to be used for central review and approval prior to the start of chemoradiation.

Follow-up of patients

A follow-up visit was required at 2 to 4 weeks after completion of chemoradiation and prior to the start of the second phase of chemotherapy after chemoradiation therapy. Thereafter, follow-up occurred at 3-month intervals for 1 year, then at 6-month intervals for 3 years, and yearly thereafter. The last date of patient follow-up was August 18, 2006. Follow-up consisted of physical examination, complete blood cell count, liver function testing, chest X-ray, and CT scanning as clinically indicated. Elevation in CA 19-9 level in and of itself was not to be considered a criterion for disease recurrence.

Statistical considerations

Survival for all patients and for patients with pancreatic head tumors were the primary end points. Secondary end points were

disease-free survival and toxicity, which was scored per the US National Cancer Institute's Common Toxicity Criteria version 2.0. All end points were prespecified in the original design of the trial and all analyses were conducted on an intention-to-treat basis. Failure for overall survival was defined as death due to any cause and was measured from date of randomization to date of death or last follow-up for censored patients. Failure for disease-free survival was defined as local, regional, or distant relapse, appearance of a second primary, or death due to any cause and measured from date of randomization to date of first failure or last follow-up for censored patients. Patients who did not have a failure for overall survival or disease-free survival were censored as of their last follow-up visit.

Patients were stratified by nodal status (uninvolved vs involved), tumor diameter (<3 cm vs ≥3 cm), and surgical margin status (negative vs positive vs unknown). The permuted block randomization method was used with patient factors balanced according to the permuted block randomization method.[1]. At an original expected accrual of 5 patients per month, 330 patients were targeted to detect a 33% reduction in the hazard rate of overall survival for the chemoradiation plus gemcitabine group compared with the chemoradiation plus fluorouracil group (increase in median survival from 18 to 27 months; hazard ratio [HR], 0.67) with 80% power and a 2-sided α level of 0.05, assuming an exponential distribution. In early 2001, based on unexpectedly rapid accrual (13 patients per month) and after approval by the US intergroup, the RTOG data monitoring committee, and the National Cancer Institute, the sample size was increased to find a smaller treatment effect with more power. Four hundred seventy analyzable patients would provide 85% power to detect a 28% decrease in the HR of overall survival (increase in median survival from 18 to 25 months; HR, 0.71) for all patients and 80% power in patients with pancreatic head lesions.

All analyses were performed using SAS version 9.1 statistical software (SAS Institute Inc.). The χ^2 tests were used to compare differences among pretreatment characteristics between treatment groups. For CA 19-9, the variable was categorized as less than 180 U/mL vs 180 U/mL or higher to be consistent with a protocol-specified CA 19-9 analysis to be performed subsequently and based on published literature.[2] The z tests were used to test for differences in binomial proportions of grade 3 toxic effects or higher (worst overall, worst nonhematologic, and worst hematologic). Overall and disease-free survival were estimated univariately using the Kaplan–Meier method and treatment groups were compared using the log-rank test.[3] Multivariate analyses were performed with Cox proportional hazard models to test for treatment differences (between groups) while adjusting for the stratification variables of nodal involvement (no vs yes), tumor diameter (<3 cm vs ≥3 cm), and margin status (negative vs positive and negative vs unknown), as well as any other variables that were imbalanced between the treatment groups. A tumor diameter of 3 cm was used for stratification based on a prior large institutional study.[4] All tests were performed at a significance level of 0.05. All variables are coded such that an HR of more than 1 indicates an increased risk for the second level of the variable and an HR of less than 1 indicates a benefit for the second level of the variable.

References

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Table S1. Baseline characteristics by dCK score for all eligible and analyzable patients

	Low (*n* = 81)		**High (*n* = 84)**		
Age					
Median	60		60		
Min–Max	35–77		36–80		
Gender	***n***	**%**	***n***	**%**	***P* values***
Male	44	54.3	47	56.0	0.83
Female	37	45.7	37	44.0	
Race					0.63
White	75	92.6	76	90.5	
African American/other	6	7.4	8	9.5	
Primary tumor location					0.046
Head	63	77.8	75	89.3	
Everything else	18	22.2	9	10.7	
KPS					0.21
60, 70, 80	27	33.3	36	42.9	
90, 100	54	66.7	48	57.1	
T-stage (surgical)					0.68
T1, T2	19	23.5	22	26.2	
T3, T4	62	76.5	62	73.8	
N-stage (surgical)					0.099
N0	32	39.5	23	27.4	
N1	49	60.5	61	72.6	
AJCC stage (5th edition)					0.097
I, II	31	38.3	22	26.2	
III, IV	50	61.7	62	73.8	
Largest tumor dimension of primary					0.51
<3 cm	34	42.0	31	36.9	
≥3cm	47	58.0	53	63.1	
Surgical margins					0.51
Complete resection/negative margins	34	42.0	36	42.9	
Complete resection/positive margins	30	37.0	25	29.8	
Complete resection/unknown margins	17	21.0	23	27.4	
Treatment arm					0.18
RT + 5-FU	48	59.3	41	48.8	
RT + gemcitabine	33	40.7	43	51.2	

**P* values from Chi-square test or Fisher Exact Test

Table S2. Baseline characteristics by dCK score for all eligible and analyzable patients, by treatment arm

	5FU arm (*n* = 89)			GEM arm (*n* = 76)		
	Low (*n* = 48)	**High (*n* = 41)**	***P* values***	**Low (*n* = 33)**	**High (*n* = 43)**	***P* values***
Age						
Median	61	61		60	60	
Min–Max	37–77	36–80		35–77	41–73	
	***n* (%)**	***n* (%)**		***n* (%)**	***n* (%)**	
Gender			0.83			0.86
Male	27 (56)	24 (59)		17 (52)	23 (53)	
Female	21 (44)	17 (42)		16 (48)	20 (47)	
Race			1.00			
White	43 (90)	37 (90)		32 (97)	39 (91)	0.38
African American/other	5 (10)	4 (10)		1 (3)	4 (9)	
Primary Tumor Location			0.99			0.0033
Head	41 (85)	35 (85)		22 (67)	40 (93)	
Everything else	7 (15)	6 (15)		11 (33)	3 (7)	
KPS categorized			0.44			0.01
60, 70, 80	19 (40)	13 (32)		8 (24)	23 (53)	
90, 100	29 (60)	28 (68)		25 (76)	20 (47)	
T-stage (surgical)			0.48			0.98
T1, T2	12 (25)	13 (32)		7 (21)	9 (21)	
T3, T4	36 (75)	28 (68)		26 (79)	34 (79)	
N-stage (surgical)			0.20			0.29
N0	19 (40)	11 (27)		13 (39)	12 (28)	
N1	29 (60)	30 (73)		20 (61)	31 (72)	
AJCC stage (5th edition)			0.28			0.20
I, II	18 (38)	11 (27)		13 (39)	11 (26)	
III, IV	30 (63)	30 (73)		20 (61)	32 (74)	
Largest tumor dimension of primary			0.75			0.13
<3 cm	16 (33)	15 (37)		18 (55)	16 (37)	
≥3cm	32 (67)	26 (63)		15 (45)	27 (63)	
Surgical margins			0.34			0.27
Complete resection/negative margins	25 (52)	17 (41)		9 (27)	19 (44)	
Complete resection/positive margins	16 (33)	13 (32)		14 (42)	12 (28)	
Complete resection/unknown margins	7 (15)	11 (27)		10 (30)	12 (28)	

**P* values from Chi-square test or Fisher's Exact Test

Table S3. Median disease-free and overall survival by dCK expression levels (low vs. high) in patients treated with gemcitabine or 5-FU.

		Low dCK (months)	**High dCK (95% CI)**	**Log-rank *P* value***
Gemcitabine (*n* = 76)	Median DFS	11.4 (7.3, 15.9)	10 (8.5, 13.6)	0.87
	Median OS	16.8 (11.5, 24.2)	18.6 (14.1, 24.1)	0.45
5-FU (*n* = 89)	Median DFS	6.7 (4.9, 11.8)	12 (9.1, 17)	0.065
	Median OS	12.9 (11.1, 17.6)	21 (15.1, 30.7)	0.012

**P* values from Chi-square test or Fisher Exact Test

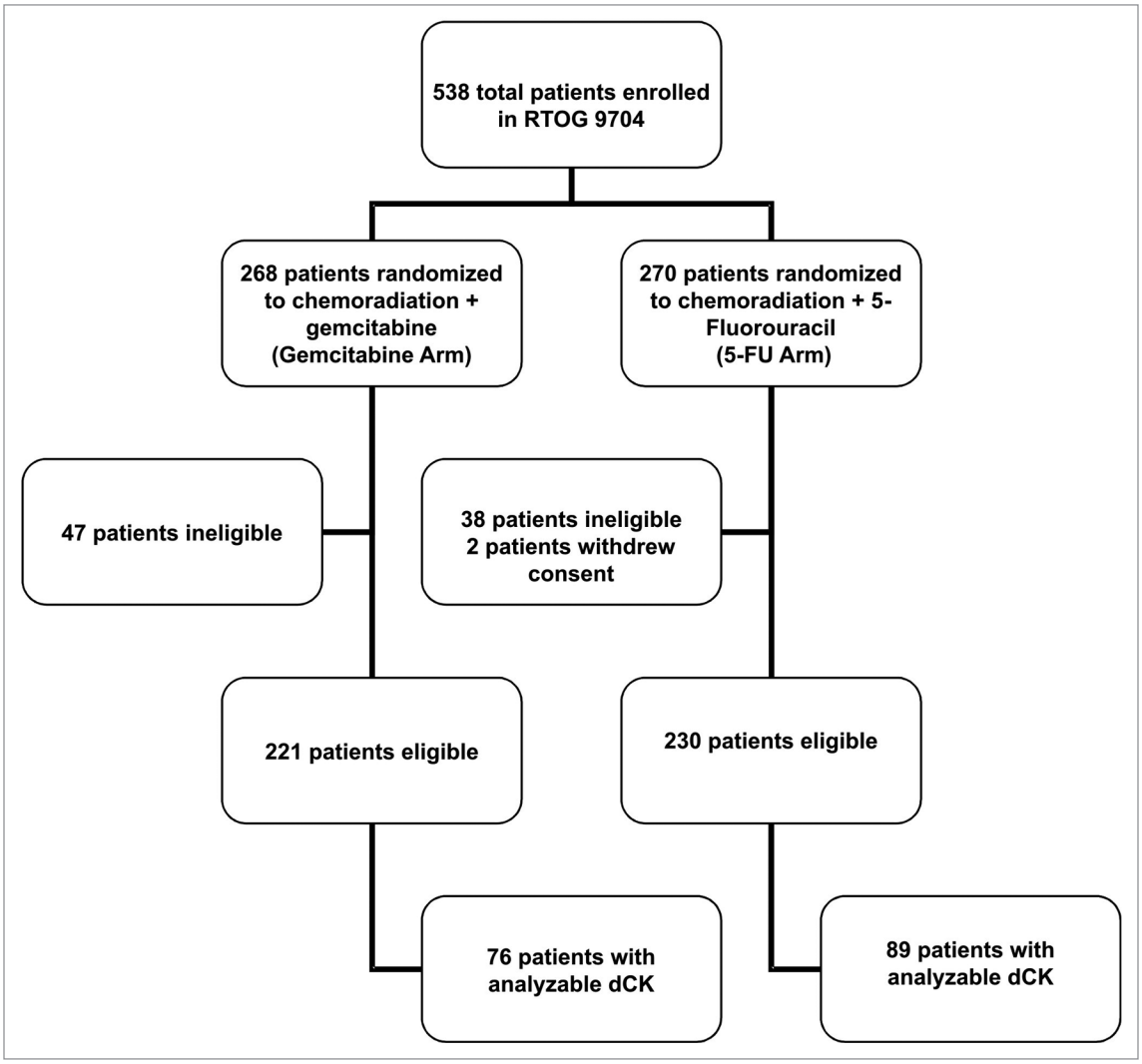

Figure S1. Flow of patients through study and protocol of RTOG 9704.

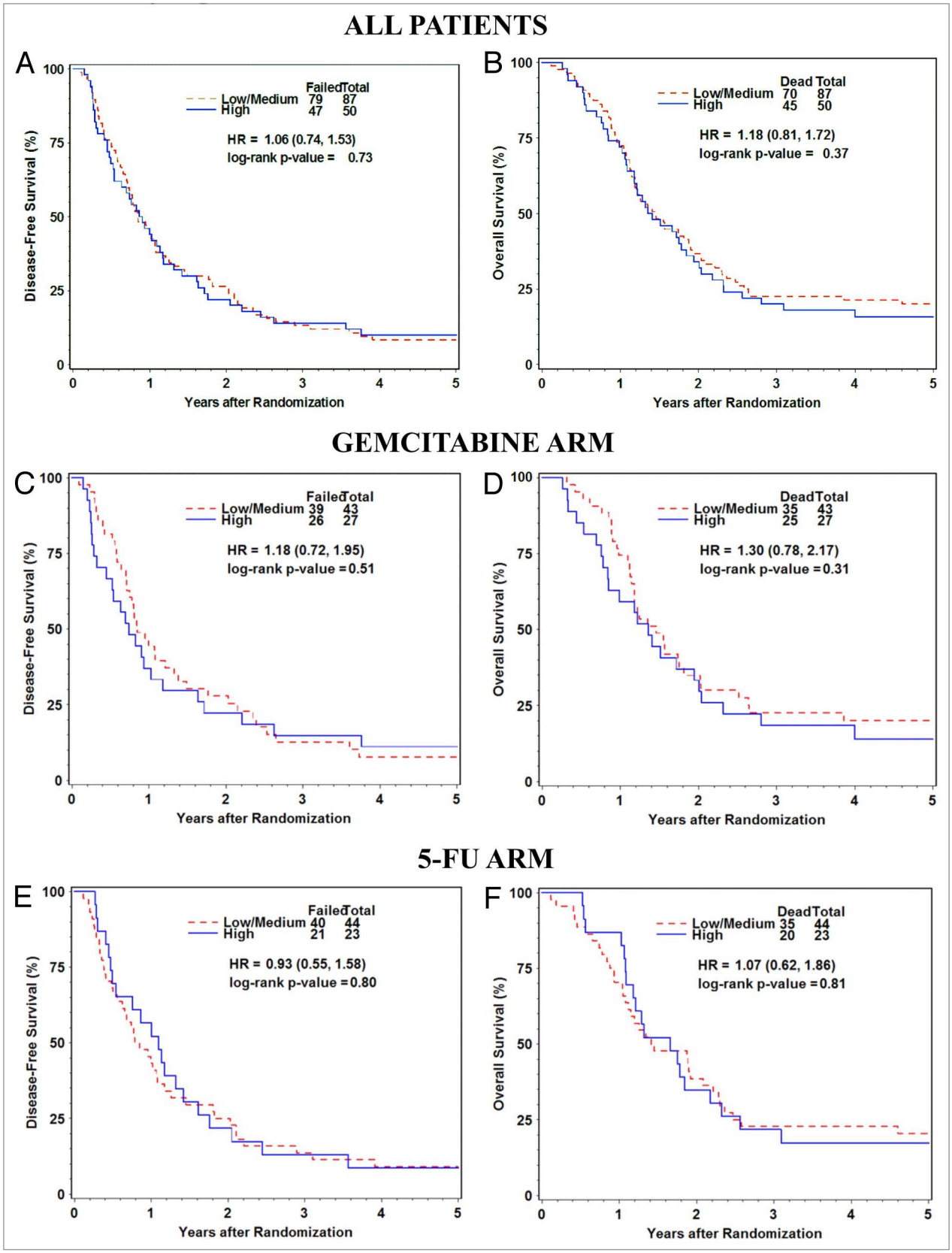

Figure S2. Survival for patients enrolled in RTOG 9704 stratified by HuR expression level. Disease-free and overall survival for all evaluable patients (**A and B**), patients on the gemcitabine arm (**C and D**) and patients on the 5-FU arm (**E and F**).

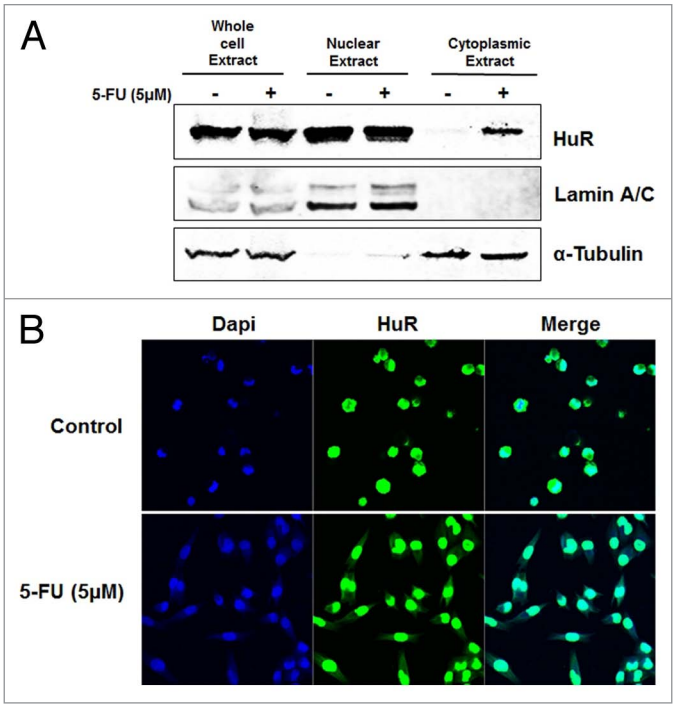

Figure S3. 5-FU causes HuR translocation. (**A**) MiaPaCa2 cells were treated with the indicated dose of 5-FU for 6 h. The immunoblot demonstrates HuR expression is significantly enhanced in cytoplasmic extracts, while unchanged in nuclear and whole cell lysates after treatment. Lamin A/C (a nuclear marker) and α-tubulin were used as protein loading controls. (**B**) 5-FU causes HuR to accumulate in the cytoplasm. MiaPaCa2 cells were treated with indicated dose of 5-FU for 12 h and fixed. Immunoflorescence was performed by staining the cells with anti-HuR antibody and DAPI was used to visualize nuclei.