Supplemental material and results

Insulin supplementation attenuates cancer-induced cardiomyopathy and slows tumor disease progression

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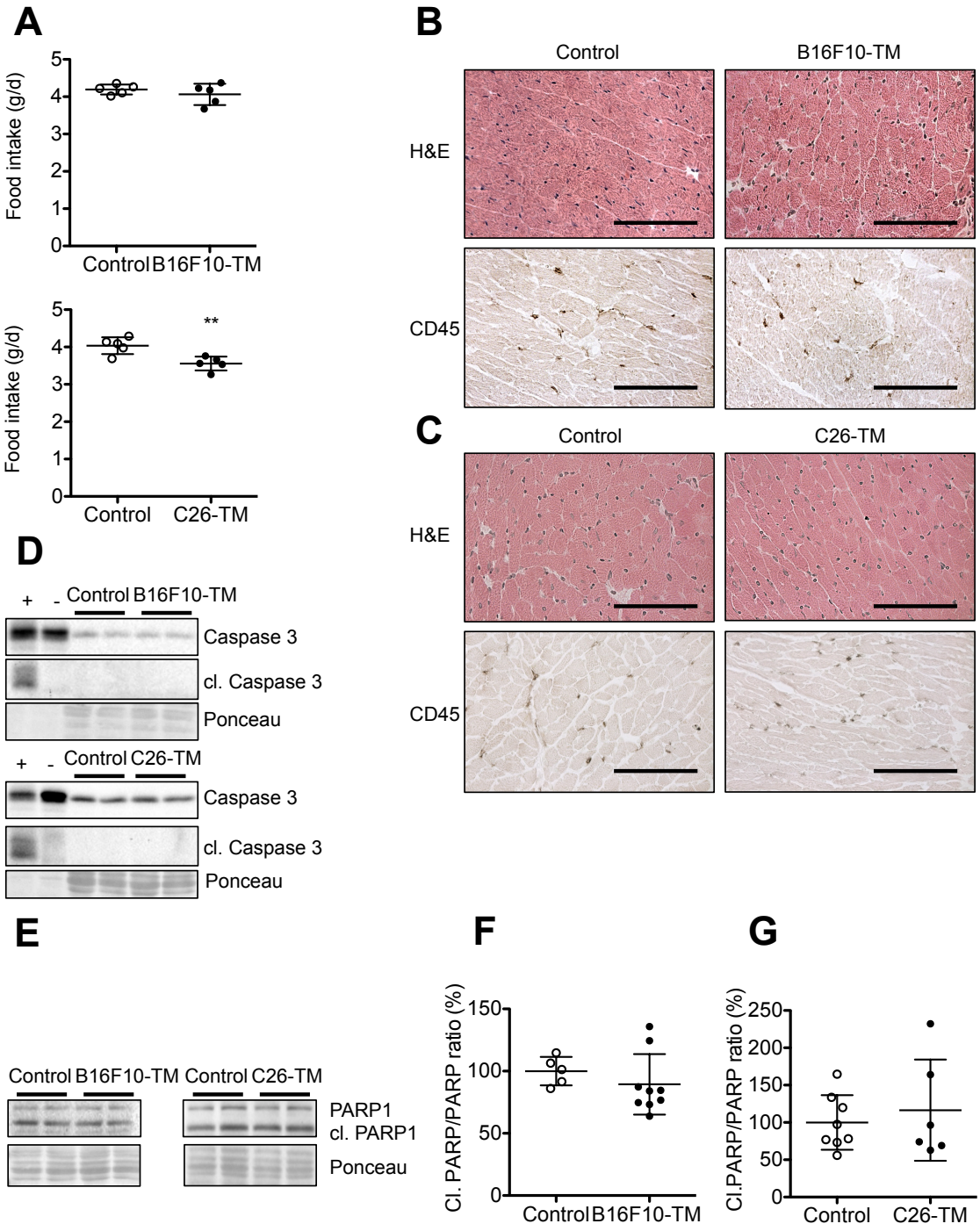

Figure S1. (A) Food intake (g/d) in B16F10-TM and C26-TM over experimental duration versus strain-matched healthy controls (n=5 each); (**B, C**) H&E staining (upper panel) and CD45 immunohistochemistry with eosin co-staining (lower panel) in B16F10-TM (n=8 vs n=9 controls) and C26-TM (n=9 vs n=9 controls for H&E, n=4 vs n=4 controls for CD45) with advanced cancer compared to respective healthy controls. Scale bars indicate 100 µm. (**D**) Western blots of Caspase 3 and cleaved Caspase 3 in B16F10-TM (n=7, controls n=5) and C26-TM (n=6, controls n=6) with advanced cancer compared to healthy controls. Jurkat cell lysates with (+) or without (-) cytochrome c treatment served as positive and negative controls for cleaved Caspase 3. (**E**) Western blots of PARP1 and cleaved PARP1 protein in LV lysates from B16F10-TM and C26-TM, both with advanced disease stage and respective healthy controls and (**F, G**) quantification of cleaved PARP/PARP protein ratio from B16F10-TM (n=9 vs control, n=5) and C26-TM (n=8 vs control, n=8). Data are depicted as

mean±SD; **P<0.01 to respective healthy controls, using two-tailed Student's unpaired t-tests.

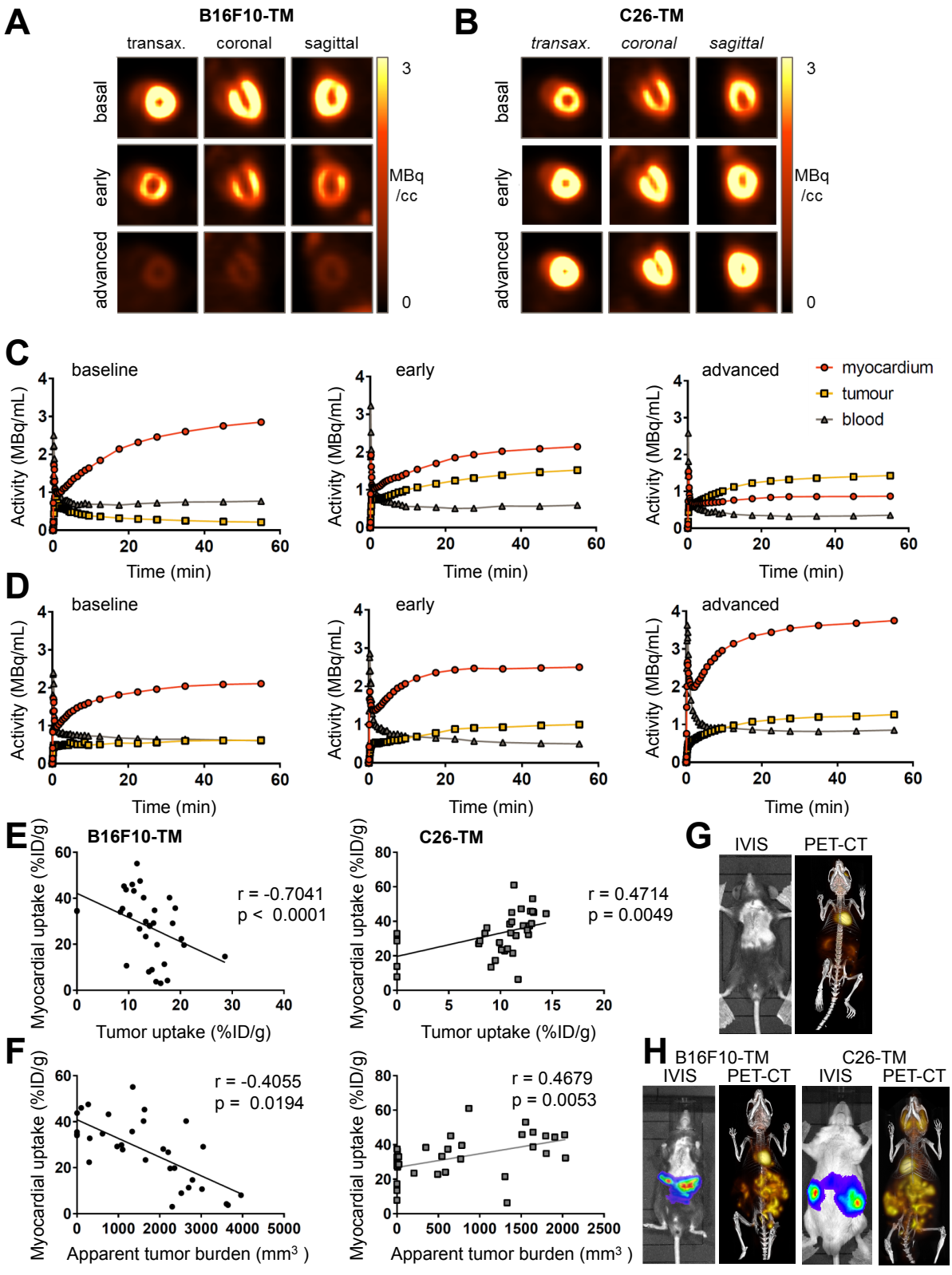

Figure S2. Representative transaxial, coronal and sagittal cardiac ^{18}F-FDG images at serial time points in (**A**) B16F10-TM and (**B**) C26-TM mice with advanced tumor disease. Mean ^{18}F-FDG time-activity curves over 60 min dynamic scan for myocardium, tumor and blood pool for (**C**) B16F10-TM (n=13) and (**D**) C26-TM mice (n=13). (**E**) ^{18}F-FDG uptake (%ID/g) in myocardium with ^{18}F-FDG uptake in tumor shows a negative correlation for B16F10-TM (r=-0.7041, P<0.0001) and a positive correlation for C26-TM mice (r=0.4714, P=0.0049). (**F**)

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Correlation of ^{18}F-FDG uptake in myocardium with ^{18}F-FDG-avid apparent tumor burden shows a similar negative correlation in B16F10-TM (r=-0.4055, P=0.0194) and positive correlation in C26-TM mice (r=0.4679, P=0.0053). Pearson product-moment correlation coefficients are calculated from aggregate comparison over the full time course for both tumor models. (**G, H**) Comparison of ^{18}F-FDG-PET-CT scan and bioimaging with IVIS before (G (C57Bl6)) and after (H) tumor implantation (in both models) with growing tumor mass on consecutive days.

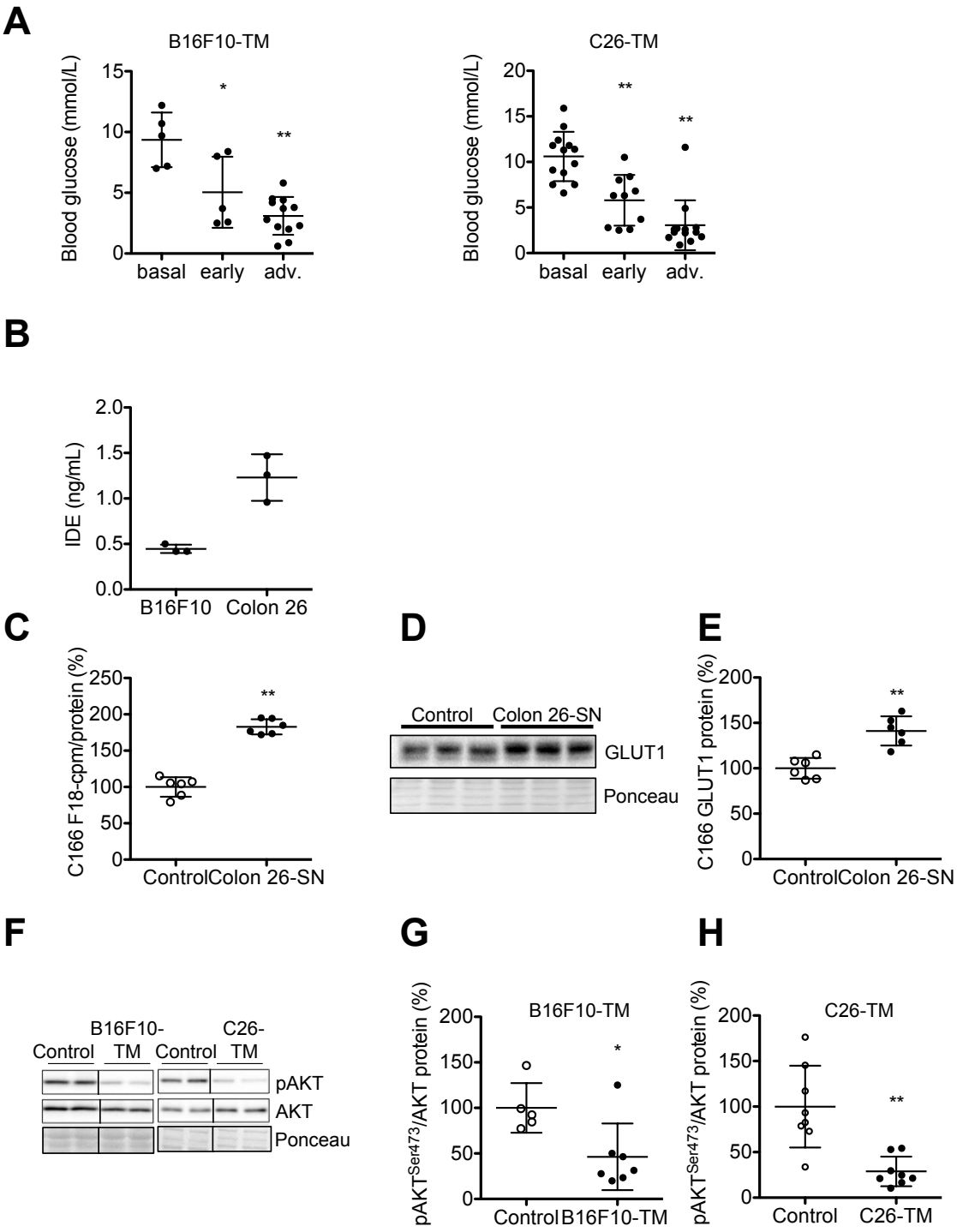

Figure S3. (A) Post-^{18}F-FDG scan blood glucose levels from B16F10-TM (n=5-12) and C26-TM (n=10-13) before tumor inoculation (basal), and at early (7 days) and advanced (14-21 days) disease stages. (**B)** Relative insulin degrading enzyme (IDE) levels in cell culture supernatants from C26 and B16F10 cells after serum-free in vitro culture for 24 h (means±SD, n=3 culture dishes per cell line were analyzed). (**C**) ^{18}F-FDG uptake of C166 cells treated with supernatants derived from Colon 26 cells (Colon 26-SN) or respective control medium (n=6 samples per group,derived from into 2 individual experiments). (**D**) Western blots of protein lysates from C166 cells treated with supernatants derived from Colon 26 cells (Colon 26-SN) or respective control medium (Control) for 24h showing GLUT1 expression and PonceauS as loading control. (**E**) Quantification of GLUT1 protein expression of C166 cells treated with supernatants derived from Colon 26 cells (Colon 26-SN) or

respective control medium (Control) for 24h (n=6 samples per group, derived from into 2 individual experiments). (**F)** Western blots showing total Akt and phosphorylation at Ser473 (pAkt) in LVs of B16F10-TM and C26-TM with Ponceau S as loading control. Samples were noncontiguous and cut for presentation as indicated. **(G, H)** Graphs with ratio of $pAkt^{S473}$/Akt in B16F10-TM (n=7) or C26-TM (n=8) with advanced disease and respective controls (n=5-8) Data are mean±SD. *P<0.05, **P<0.01 vs basal or respective control using two-tailed Student's unpaired t-tests or using one-way ANOVA with Bonferroni post hoc tests.

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Table S1. Clinical data from patients and healthy controls

		Tumor patients	Controls
N		14	13
Age	years	59.05 (51.48-69.23)	56 (54-61.5)
Sex	male %	50	77
BMI	[kg/m²]	21.7 (19.63-22.63)	N/A
weight loss	[kg]	9 (5-18.5)	N/A

Data are presented as the percentage or the median (25^{th}-75^{th} percentile). Weight loss is indicated over a period of 12 months. BMI = Body Mass Index. N/A = not available. **Etiology of cancer in these patients:** Hepatocellular (36%), Colo-rectal (29%), Gastric (14%), Oesophagal (14%), other (7%). Only tumor patients with a body weight loss >5% within the last 12 months before sample collection were included.

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Table S2. Primers

mRNA	Sense primers (5′ to 3′)	Antisense primers (5′ to 3′)
18S rRNA	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
ANP	GCCGGTAGAAGATGAGGTCA	GGGCTCCAATCCTGTCAATC
Atrogin1	CTTCTCGACTGCCATCCTGG	GTTCTTTTGGGCGATGCCAC
CatL	CCTATGAAGCGAAGGACGGA	TTCACGACAGGATAGCTGGC
Irs-2	CCATCGATGTGAGAGGCGAG	GGCGATGGGGCTGGTAG
LC3b	CATGCCGTCCGAGAAGACCT	TCGCTCTATAATCACTGGGATCTTG
Myh6	GGAAGAGCGAGCGGCGCATCAAGG	GTCTGCTGGAGAGGTTATTCCTCG
Myh7	CAAGTTCCGCAAGGTGC	AAATTGCTTTATTCTGCTTCCAC
MuRF1	GAGGGCCATTGACTTTGGGA	CCAGAGCGTGTCTCACTCAT