Electronic Supplementary Material (ESM)

ESM Methods

Matching Process: A propensity score [1], the conditional probability of being a donor that spent the least amount of time in the hospital, given the four covariates, was calculated for all non-diabetic donors with the exception that a multinomial logistic region model was used to account for the 3 hospitalization time groups. The logit of the propensity score was used to match donors across all three groups in a 1:1:1 ratio using a randomly sorted nearest-available-neighbor matching method without replacement. Although an optimal caliper width equal to 0.20 of the standard deviation of the logit value has been shown to be ideal in a 1:1 matching [2], no such studies have been performed in 1:1:1 scenarios. We used two times the caliper width from 1:1 matching, in line with a suggestion from authors of the Pharmacoepi Toolbox [3], a collection of freely available SAS macros adopted for implementation of this analysis.

Image Acquisition and Processing: The stained slides were digitalized at an absolute magnification of 20x using the Aperio SC2 slide scanner (Leica). These high-quality whole slide images were analyzed using the HALO Next Generation quantitative image analysis platform (Indica Labs). Ducts, connective and fat tissues, region with edge artifacts, and nonspecific staining were excluded from analysis by applying the tissue classification algorithm (Indica Labs) and manual annotations to each tissue section. The image analysis based on red, blue, green (RBG) spectra was used to detect all cells by counterstaining with hematoxylin (blue), 3,3’-Diaminobenzidine (DAB, brown) and fast red. The quantification algorithm configured for dual-stain immunohistochemistry (IHC)

was designed based on Indica Lab's CytoNuclear IHC Quantification tool. The algorithm detected single or double IHC positivity and calculated percentage cellularity (% positive cells/all nucleated cells) per tissue section.

Analysis Variables: Pancreas region was treated as a repeated measurement variable because staining was done using tissue sections across the entire pancreas (i.e., head, body, and tail regions). Coding of age as a categorical variable was based on data by Gregg et al showing differences in Ki67/insulin staining in development, neonates, children, adolescents, and adults [4]. To avoid having a group with only 1 donor, and to minimize the number of small groups, consolidation of the categories used by Gregg et al was performed as follows: development (n=1), neonates (n=4), and children (n=6) as group 1 (<12 years of age, n=11 total), adolescent or youth as group 2 (≥12 to <20 years of age, n=5) and adults as group 3 (≥20 years of age, n=23).

Power and Sample Size: Pilot data, from three donors not used in this study and of the same age group, were generated to estimate the residual variance in measurements of each primary outcome variable. The standard deviation was found to be 1.98% for $CD45^+$, 0.55% for $CD68^+$, and 0.74% for $Ki67^+$ cells.

Initial estimates of cell frequencies for leukocytes from donors in the <3 day hospitalization group were determined using previously published data [5, 6] and a series of calculations and found to be 1.96% for both $CD45^+$ and $CD68^+$. The magnitude of the differences in the number of cells counted by In'tVeld and colleagues [7] were used to

estimate initial leukocyte cell frequencies for donors in the (3,6) day (1.96% for $CD45^+$, 2.94% for $CD68^+$) and ≥6 day (5.88% for both $CD45^+$ and $CD68^+$) hospitalization groups.

For Ki67, cell frequency data was obtained from In'tVeld et al. [7], who estimated that levels were ≤0.1% in 262 (72.2%) of screened donors; therefore, 0.1% was used as an initial estimate in the <3 day hospitalization group. The percentage of Ki67 in the (3,6) day hospitalization group was selected to be 0.6%, because this had been previously defined [7] as being the minimum value for organ donors with high levels of beta-cell replication. Likewise, in the ≥6 day hospitalization group, the percentage of $Ki67^+$ cells was selected to be 1.59%, a calculated mean of all organ donors previously defined [7] as being in the group with high levels of beta-cell replication.

Given the sample size of 39 donors (i.e. 13 trios), a two-sided $\alpha=0.05$, and using an ANOVA test, there is 99% power to detect a) a threefold increase in mean $CD45^+$ cell percentages in the ≥6 day hospitalization group compared to the <3 day hospitalization group, no differences were expected between the <3 and (3,6) day hospitalization groups; b) 1.5 and 3 fold increase in mean CD68 percentages in the (3,6) and ≥6 day hospitalization groups, respectively; and c) six and 16 fold increases in mean $Ki67^+$ cell percentages in the (3,6) and ≥6 day hospitalization groups, respectively. Sphericity (i.e., satisfaction of the Huynh and Feldt condition) [8] and a compound symmetry variance-covariance structure were both assumed for these calculations. Additionally, leukocyte or $Ki67^+$ cell percentages were assumed to be constant, irrespective of the region of pancreas sampled.

ANOVA Model Details: Association between hospitalization and age groups was indicated if $p<0.05$ and Kendall's tau-b <-0.30 or >0.30. Pancreas region was treated as a repeated measures variable. The model assumption of sphericity (satisfaction of the Huynh and Feldt condition) [8], was evaluated by using the Mauchly criterion test [9]. If the chi-square p-value<0.05 (i.e., model violation), then the Greenhouse-Geisser (GG; [10]) corrected univariate p-values were used for within subjects effects; otherwise, unadjusted univariate p-values were used for both between and within subjects effects, including interaction terms. If a statistically significant main effect was present, in the absence of an interaction, point estimates and 95% confidence intervals (CIs) of least square mean differences between individual levels were calculated; *p*-values and CIs were adjusted for these multiple comparisons using the method of Tukey-Kramer, unless otherwise noted.

ESM Table 1. Characteristics of matched non-diabetic individuals with variation in length of hospital stay prior to pancreas donation

Name	(%) or Mean (± 1 SD)[a]					
	n	<3 days	n	≥3 to <6 days	n	≥ 6 days
Age at Death (yrs.)[b]	13	20.3(±11.2)	13	23.1(±17.0)	13	23.8(±16.1)
Sex[b]						
Female	3	23%	4	31%	7	54%
Male	10	77%	9	69%	6	46%
BMI (kg/m2)[b]	13	24.3(±6.0)	13	23.9(±5.4)	13	24.0(±5.9)
Ethnicity[b]						
Caucasian	10	77%	12	92%	10	77%
African American	2	15%	0	0%	2	15%
Hispanic/Latino	1	8%	1	8%	1	8%
Cause of Death						
Anoxia	5	38%	4	30%	3	24%
Cerebrovascular/Stroke	2	16%	1	8%	4	30%
Head Trauma	5	38%	8	62%	5	38%
Other[c]	1	8%	0	0%	1	8%
C-peptide[d](nmol/L)	12	1.9(±1.6)	13	1.5(±1.8)	9	2.3(±1.9)
Pancreas Transport Time[e] (hrs.)	13	14.9(±6.1)	13	16.2(±9.9)	13	11.0(±7.9)
Medications[f]						
Anesthetics	3	23%	5	38%	6	46%
Antibiotics	12	92%	12	92%	6	46%
Cardiovascular	12	92%	10	77%	11	85%
Hormonal	10	77%	8	62%	8	62%
Fluids/Electrolytes	4	31%	1	8%	3	23%
Other[g]	12	92%	13	100%	13	100%

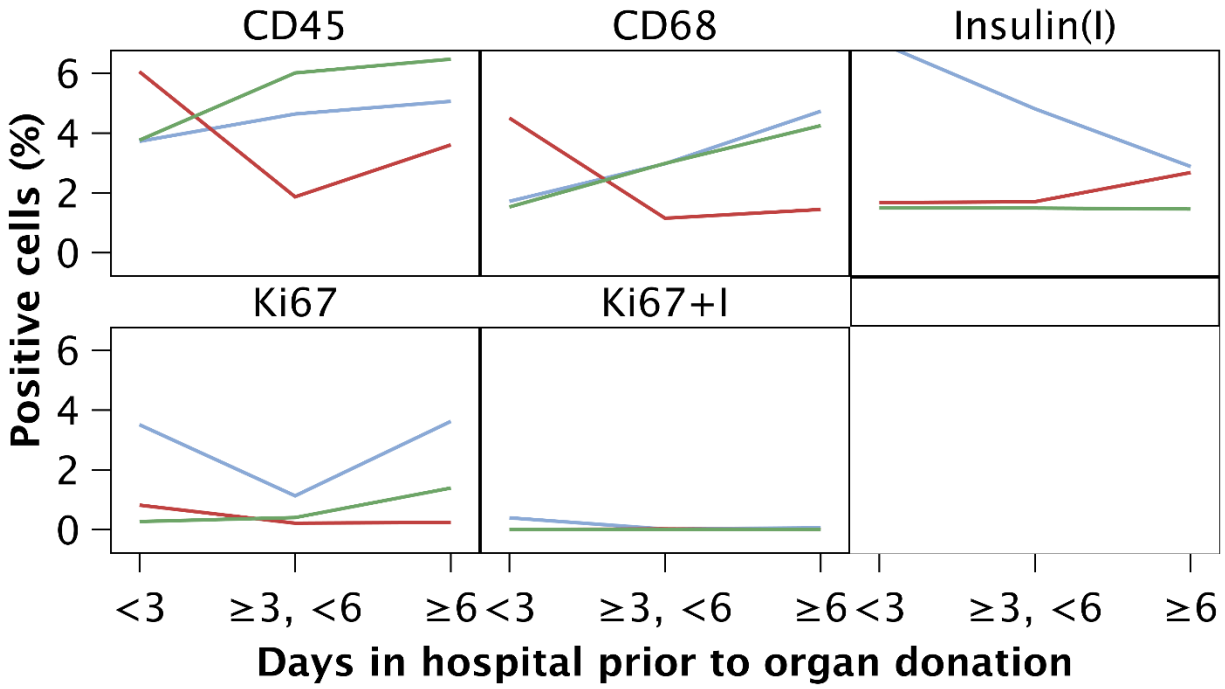
[a]Number and % are reported for all categorical variables. Use of mean (± 1 SD) for continuous variables.
[b]Factors used for matching; As such, age (p=0.818, ANOVA), sex (p=0.339, Freeman-Halton extension of Fishers exact), BMI (p=0.976, ANOVA), and ethnicity (p=0.767, Freeman-Halton extension of Fishers exact).
[c]Grouping in this category included 2 donors with cause of death originally noted as pulmonary hypoplasia (1) and respiratory distress/failure (1).
[d]Taken within 24 hrs. prior to or at death
[e]Indicates time from aortic cross-clamp to laboratory receipt (p=0.2491, ANOVA).
[f] Number of individuals (and % of total) having received medications less than 24 hours prior to organ donation (i.e. aortic cross-clamp). List reduced from 114 individual medications plus 11 categories or specific drugs and placed into 6 manageable classes based on mechanism of action.
[g]Medications with wide-ranging action placed into this category.

ESM Table 2. Length of hospitalization prior to organ donation in relation to the percentages of cells stained in the pancreas.

IHC Stain	Pancreas Region	% Stained Cells[a,b]			*p*-value[d]
		<3 days	≥3 to <6 days	≥6 days	
CD45	Head	3.98±1.01	3.64±0.97	4.86±1.21	0.902
	Body	4.22±1.15	4.17±1.11	4.61±1.38	
	Tail	5.35±1.47	4.71±1.42	5.67±1.76	
	Overall[c]	4.52±1.17 (2.12, 6.91)	4.17±1.13 (1.87, 6.48)	5.05±1.40 (2.19, 7.91)	0.889
CD68	Head	2.12±0.80	2.12±0.77	3.44±0.95	0.638
	Body	2.82±0.82	2.59±0.79	3.19±0.98	
	Tail	2.82±0.99	2.42±0.95	3.81±1.18	
	Overall[c]	2.59±0.83 (0.89, 4.28)	2.37±0.80 (0.73, 4.01)	3.48±1.00 (1.44, 5.51)	0.677
Insulin	Head	3.68±0.85	2.60±0.82	2.26±1.01	0.504
	Body	2.97±0.66	2.23±0.63	2.07±0.79	
	Tail	3.54±0.73	3.19±0.71	2.70±0.88	
	Overall[c]	3.40±0.73 (1.91, 4.88)	2.67±0.70 (1.24, 4.10)	2.34±0.87 (0.57, 4.12)	0.619
Ki67	Head	1.49±0.85	0.50±0.82	1.94±1.01	0.530
	Body	1.71±0.82	0.67±0.80	1.75±0.99	
	Tail	1.41±0.66	0.59±0.63	1.58±0.79	
	Overall[c]	1.54±0.77 (0.04, 3.11)	0.59±0.75 (0.93, 2.11)	1.76±0.92 (-0.13, 3.64)	0.548
Dual (Ki67 + Insulin)	Head	0.23±0.10	0.02±0.10	0.03±0.12	0.216
	Body	0.08±0.03	0.01±0.03	0.02±0.04	
	Tail	0.10±0.04	0.03±0.04	0.02±0.05	
	Overall[c]	0.14±0.06 (0.02, 0.26)	0.02±0.06 (0.10, 0.14)	0.03±0.07 (0.12, 0.17)	0.305

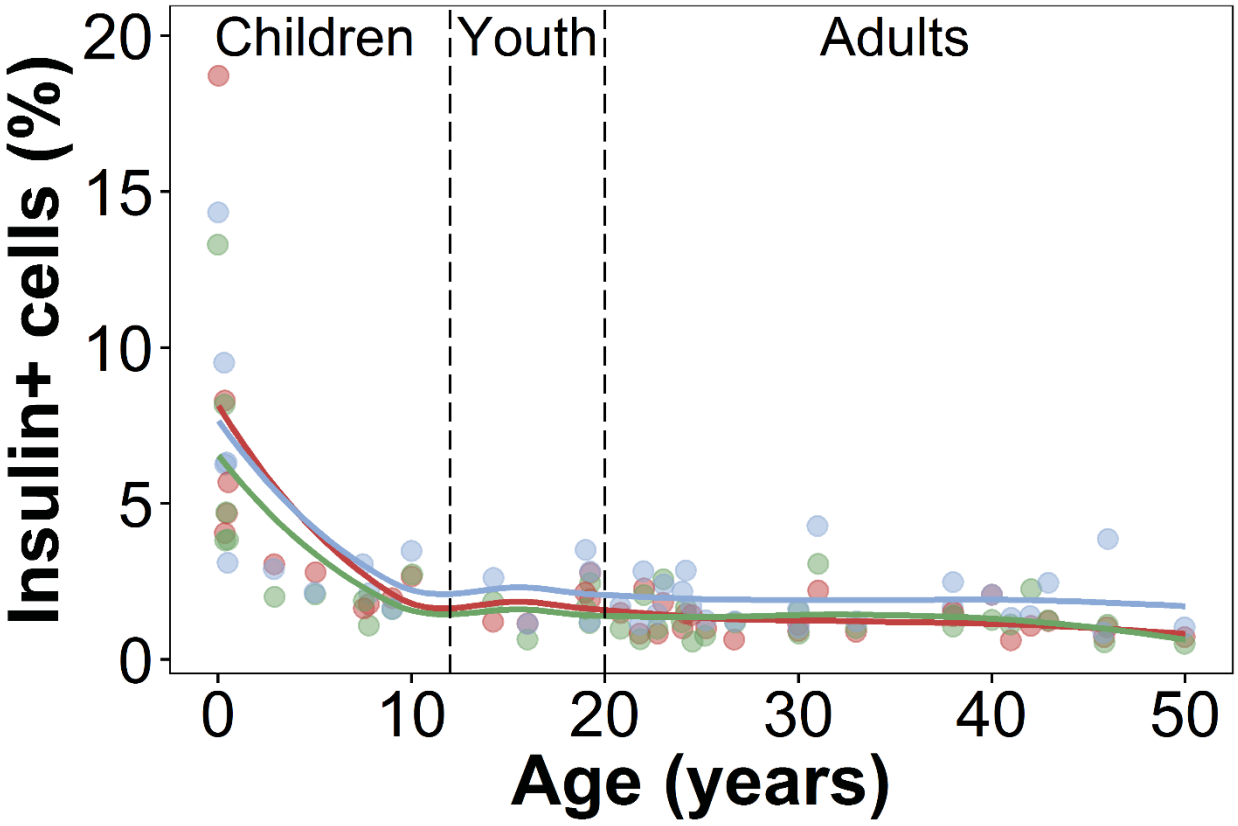
[a] Calculated as the percentage of positive cells over all nucleated cells per tissue section. Measurements were taken from the head, body, and tail of each donor.
[b] Least Squares Means (LS Means) and Standard Error (SE) values reported; Means represent readings from 13 donors per hospitalization group (n=39 total).
[c] Overall numbers represent grand mean values. 95% CI in parenthesis.
[d] See statistical analysis for details on p-value calculations.

ESM Figure 1. Simple Main Effects of Hospitalization and Age on Percentage of Cells.



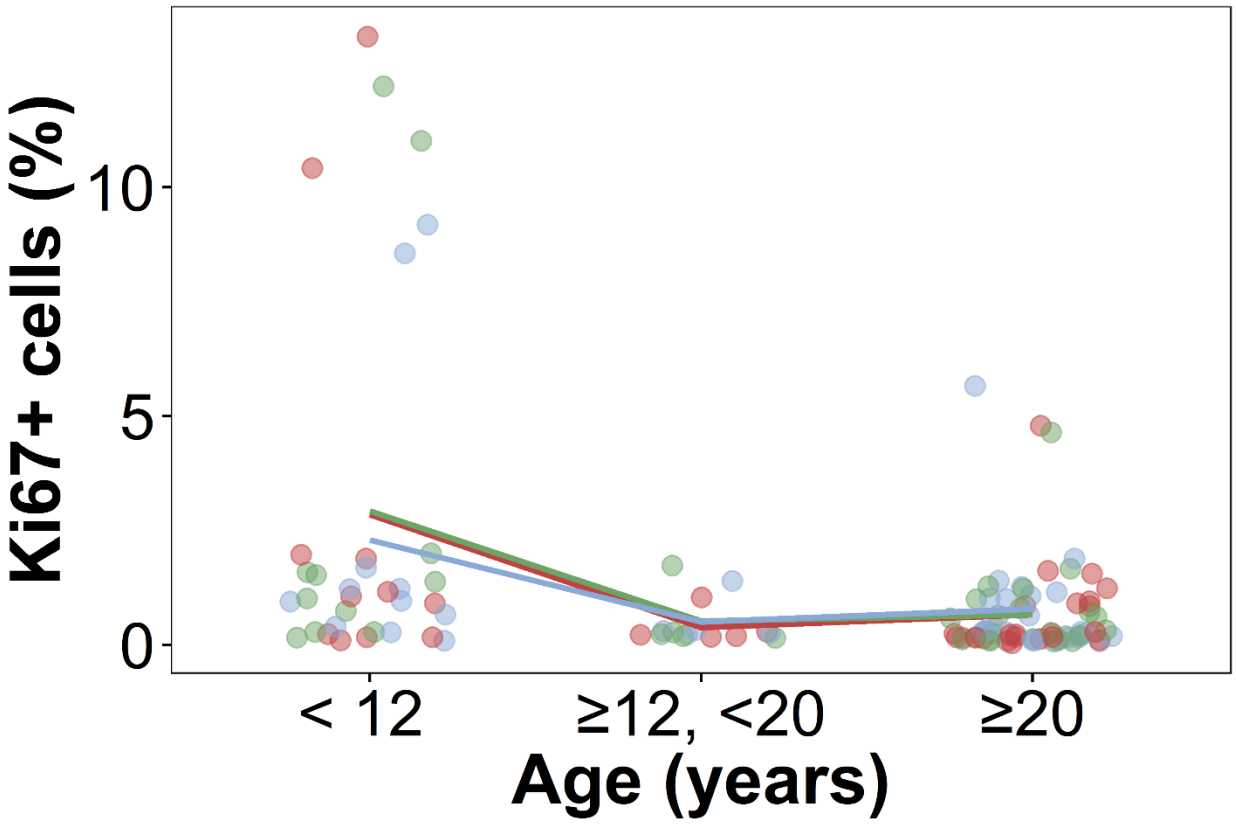
The interaction of hospitalization time with age group for organ donors <12 (blue, group 1), ≥12 to <20 (red, group 2), and ≥20 (green, group3) years of age. No statistically significant interactions were found when staining with $CD45^+$ (p=0.600), $CD68^+$ (p=0.382), $Insulin^+$ (p=0.339), $Ki67^+$ (p=0.800), or $Ki67^+/insulin^+$ (p=0.183).

ESM Figure 2. Distribution of Insulin$^+$ Cells by Age Group and Pancreas Region



Samples from the head (red), body (green), and tail (blue) region of the pancreas were obtained from each donor ((39 donors, 117 data points total). Lines were determined using the locally weighted smoothing (LOESS) method. By ANOVA, statistically significant differences in pancreas region (p=0.006) and age group (p=0.001) were found. See main text for post-hoc comparisons.

ESM Figure 3. Ki67+ cells by Age Group and Pancreas Region.



Head (red), body (green), and tail (blue) data from each donor are plotted in circles (39 donors, 117 data points total). Simple main effects of age group by pancreas region plotted using mean values to form each line (overall p=0.027). See main text for post-hoc comparisons.

ESM References

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