SUPPLEMENTARY DATA

Supplementary Table S1. Characteristics of human islet donors used in these studies

Donor number	Diagnosed diabetes	Sex	Age (years)	BMI	Ethnicity	Cause of Death	Experiments performed
1	No	F	45	26.6	White	Cerebrovascular/stroke	Fig 6 A-C
2	No	F	56	33.4	Black or African American	Cerebrovascular/stroke	Fig 6 A-C
3	No	M	55	28.5	White	Anoxia	Fig 6 A-I, Fig 7 A-F
4	No	F	51	22.5	Asian	Cerebrovascular/stroke	Fig 6 A-I, Fig 7 A-F
5	No	M	54	21.7	White	Anoxia	Fig 6 A-I, Fig 7 A-F

SUPPLEMENTARY DATA

Supplementary Table S2. Number of cells counted for each experiment

Figure panels	Type of cells	Experimental conditions	Outcomes	number of biological replicates	Total number of cells counted	Number of cells counted (mean +/- SE)
1B-1C	mouse	5mM glu	BrdU, gH2AX, predicted, observed	6	14295	2382 +/- 471
2A-2C	mouse	5mM, young	BrdU, gH2AX, predicted, observed	5	9134	1827 +/- 134
2A-2C	mouse	15mM, young	BrdU, gH2AX, predicted, observed	10	18473	1847 +/- 290
2A-2C	mouse	5mM, old	BrdU, gH2AX, predicted, observed	4	11009	2752 +/- 642
2A-2C	mouse	15mM, old	BrdU, gH2AX, predicted, observed	4	11566	2892 +/- 342
2D-2F	mouse	5mM, cre, young	BrdU, gH2AX, predicted, observed	5	9203	1841 +/- 181
2D-2F	mouse	5mM, D2, young	BrdU, gH2AX, predicted, observed	5	9589	1918 +/- 367
2D-2F	mouse	5mM, cre, old	BrdU, gH2AX, predicted, observed	4	8928	2232 +/- 583
2D-2F	mouse	5mM, D2, old	BrdU, gH2AX, predicted, observed	4	8937	2234 +/- 245
2G-2I	mouse	15mM, cre, young	BrdU, gH2AX, predicted, observed	5	9653	1931 +/- 302
2G-2I	mouse	15mM, D2, young	BrdU, gH2AX, predicted, observed	5	12306	2461 +/- 434
2G-2I	mouse	15mM, cre, old	BrdU, gH2AX, predicted, observed	4	10947	2737 +/- 360
2G-2I	mouse	15mM, D2, old	BrdU, gH2AX, predicted, observed	4	10341	2585 +/- 625
2J-2L	mouse	5mM, veh	BrdU, gH2AX, predicted, observed	3	5347	1782 +/- 280

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2J-2L	mouse	5mM, harmine	BrdU, gH2AX, predicted, observed	3	3626	1209 +/- 193
2J-2L	mouse	15mM, veh	BrdU, gH2AX, predicted, observed	3	4696	1565 +/- 19
2J-2L	mouse	15mM, harmine	BrdU, gH2AX, predicted, observed	3	3737	1246 +/- 105
3A-3C	mouse	15mM, ctrl, young	BrdU, gH2AX, predicted, observed	7	9978	1425 +/- 233
3A-3C	mouse	15mM, mito, young	BrdU, gH2AX, predicted, observed	6	4239	707 +/- 378
3A-3C	mouse	15mM, ctrl, old	BrdU, gH2AX, predicted, observed	4	11566	2892 +/- 342
3A-3C	mouse	15mM, mito, old	BrdU, gH2AX, predicted, observed	4	10192	2548 +/- 400
3D-3F	mouse	15mM, ctrl, old	BrdU, gH2AX, predicted, observed	4	11566	2892 +/- 342
3D-3F	mouse	15mM, UV, old	BrdU, gH2AX, predicted, observed	4	10141	2535 +/- 278
5A	mouse	15mM, +BrdU, young	gH2AX	7	9978	1425 +/- 233
5A	mouse	15mM, no BrdU, young	gH2AX	4	8606	2152 +/- 595
5A	mouse	15mM, +BrdU, old	gH2AX	4	11566	2892 +/- 342
5A	mouse	15mM, no BrdU, old	gH2AX	4	14538	3635 +/- 926
5C	mouse	15mM, X, young	gH2AX	5	7570	1514 +/- 365
5C	mouse	15mM, Y, young	gH2AX	7	9978	1425 +/- 233
5C	mouse	15mM, Z, young	gH2AX	5	7515	1503 +/- 371

5C	mouse	15mM, X, old	gH2AX	4	11203	2801 +/- 355
5C	mouse	15mM, Y, old	gH2AX	4	11566	2892 +/- 342
5C	mouse	15mM, Z, old	gH2AX	4	10111	2528 +/- 365
5D	mouse	5mM, D2, + BrdU, young	BrdU, gH2AX	3	5423	1808 +/- 286
5D	mouse	5mM, D2, no BrdU, young	BrdU, gH2AX	3	3663	1221 +/- 208
5D	mouse	5mM, D2, + BrdU, old	BrdU, gH2AX	3	12684	4228 +/- 196
5D	mouse	5mM, D2, no BrdU, old	BrdU, gH2AX	3	10600	3533 +/- 252
5E	mouse	15mM, D2, + BrdU, young	BrdU, gH2AX	3	3700	1233 +/- 202
5E	mouse	15mM, D2, no BrdU, young	BrdU, gH2AX	3	5762	1921 +/- 228
5E	mouse	15mM, D2, + BrdU, old	BrdU, gH2AX	3	8296	2765 +/- 102
5E	mouse	15mM, D2, no BrdU, old	BrdU, gH2AX	3	8291	2764 +/- 279
5F-5G	mouse	15mM, lacZ, young	pHH3	4	8366	2092 +/- 214
5F-5G	mouse	15mM, D2, young	pHH3	4	8244	2061 +/- 302
6A-6C	human	5mM	BrdU, gH2AX, predicted, observed	4	4729	1182 +/- 301
6A-6C	human	15mM	BrdU, gH2AX, predicted, observed	5	3817	763 +/- 162
6D-6F	human	5mM, cre	BrdU, gH2AX, predicted, observed	3	2651	884 +/- 227
6D-6F	human	5mM, D2	BrdU, gH2AX, predicted, observed	3	3571	1190 +/- 196

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6G-6I	human	15mM, cre	BrdU, gH2AX, predicted, observed	3	2615	872 +/- 208
6G-6I	human	15mM, D2	BrdU, gH2AX, predicted, observed	3	4271	1424 +/- 261
7A-7C	human	15mM, ctrl	BrdU, gH2AX, predicted, observed	5	3817	763 +/- 162
7A-7C	human	15mM, mito	BrdU, gH2AX, predicted, observed	5	3207	641 +/- 303
7D-7F	human	15mM, ctrl	BrdU, gH2AX, predicted, observed	5	3817	763 +/- 162
7D-7F	human	15mM, UV	BrdU, gH2AX, predicted, observed	3	2971	990 +/- 247

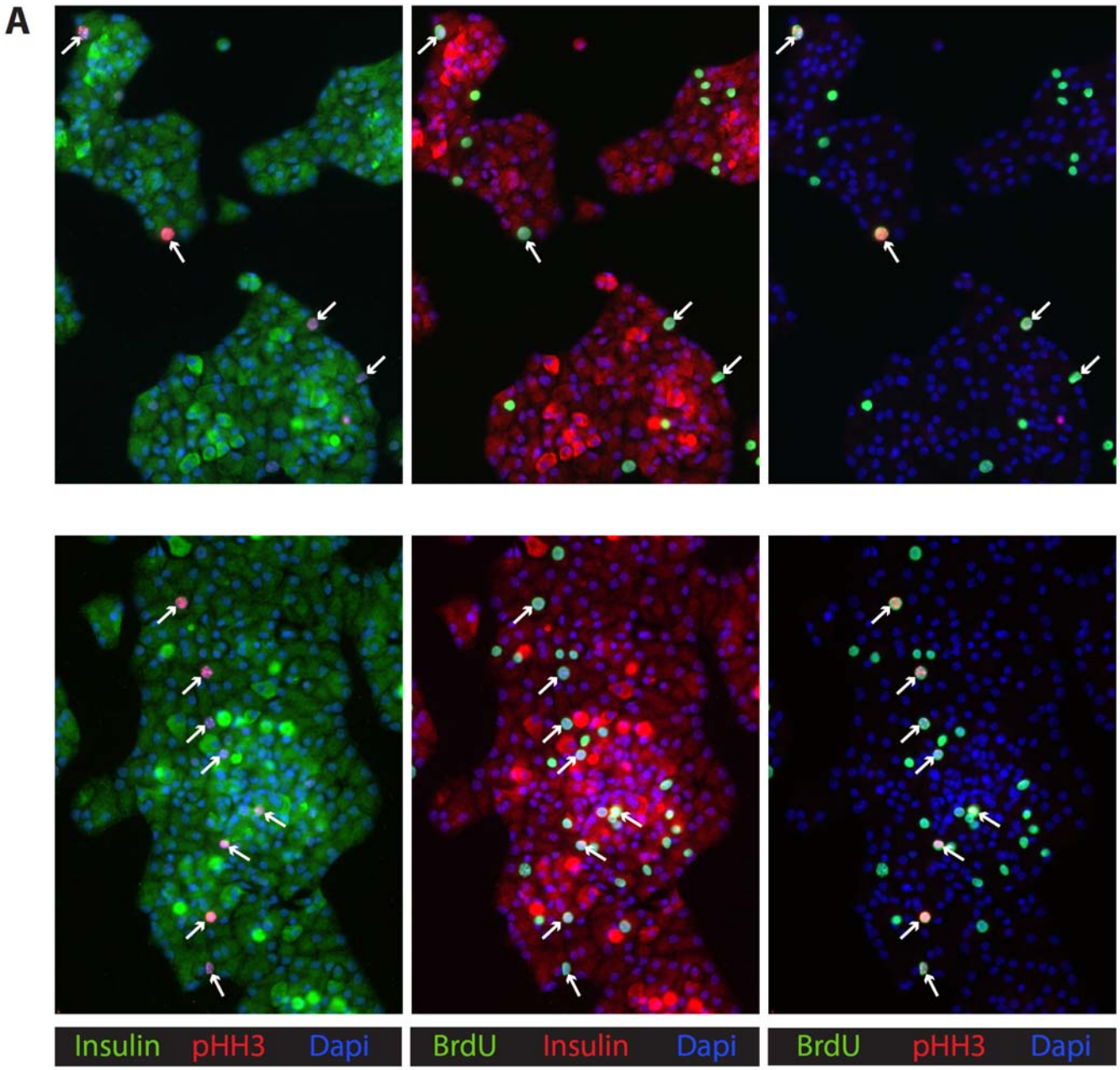
SUPPLEMENTARY DATA

Supplementary Table S3. Putative explanations for beta cell co-labeling with BrdU and DNA damage marker gH2AX, implications, and an approach to testing each hypothesis.

Explanation	Possible mechanism if true	Implications if true	How to test
DNA damage causes BrdU incorporation	DNA damage repair results in incorporation of BrdU nucleotide	BrdU incorporation does not reflect cell cycle entry in cells with DNA damage.	Induce DNA damage, test for increased frequency of BrdU labeling. Observe pattern of BrdU label.
Brdu exposure causes DNA damage	Incorporation of the BrdU nucleotide leads to a DNA damage response	BrdU incorporation reflects cell cycle entry but incurs toxicity. BrdU toxicity may impact cell cycle completion.	Test for increased DNA damage in the presence of BrdU compared with DNA damage in the absence of BrdU
An upstream process causes both DNA damage and BrdU incorporation	Many possible mechanisms. Most likely is that proliferative signals, DNA replication or other cell cycle related process also induces DNA damage	BrdU incorporation reflects cell cycle entry. Cells may not complete the cell cycle. Proliferation is a dangerous process for beta cells.	Test whether proliferative conditions increase the fraction of gH2AX-labeled cells.
DNA damage and BrdU incorporation are unrelated but occasionally co-occur stochastically	Cells occasionally co-label with BrdU and gH2AX by random chance	BrdU incorporation reflects cell cycle entry.	Test whether the observed BrdU-gH2AX co-labeling frequency matches the predicted frequency based on prevalence of the individual labels

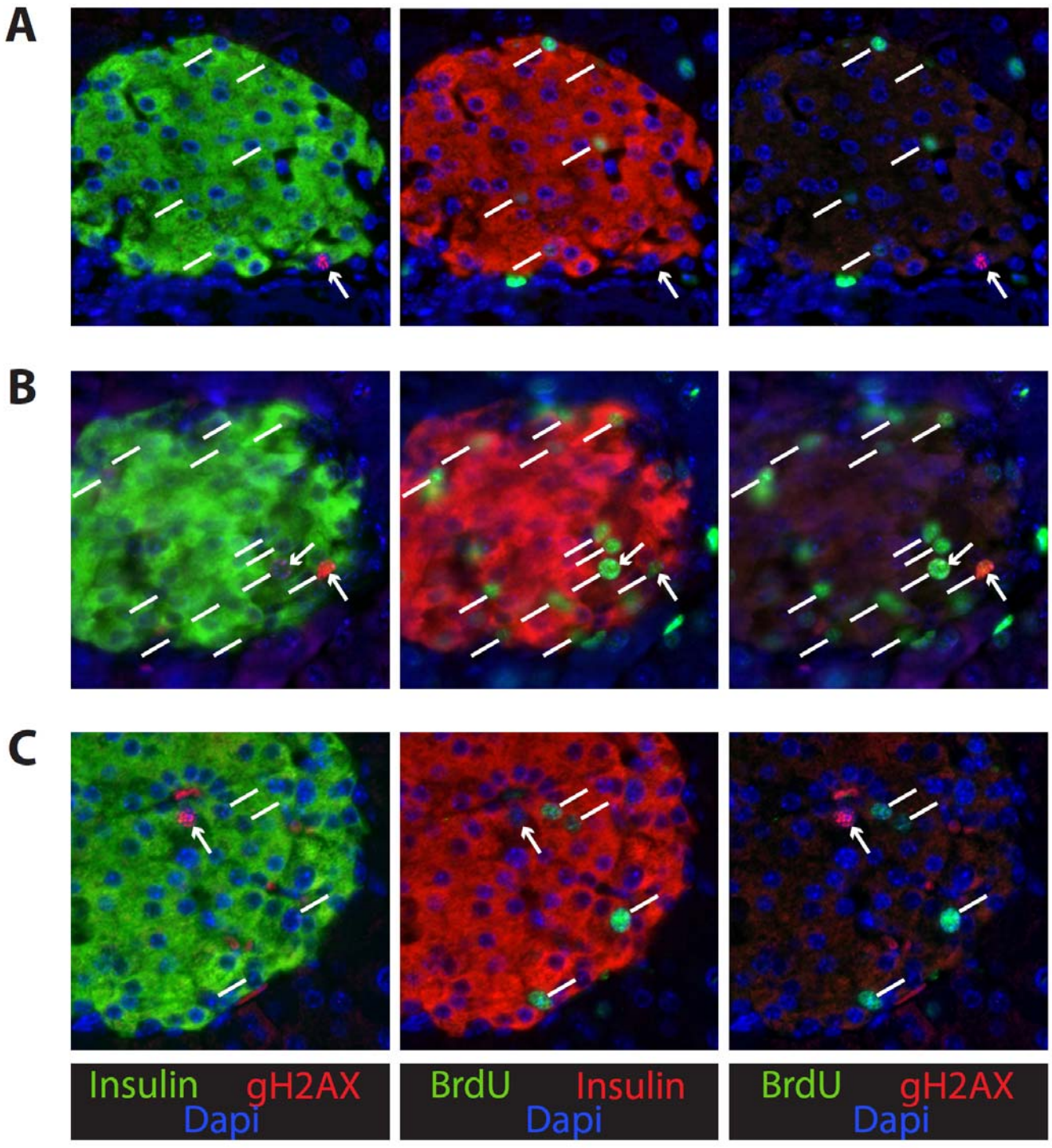
SUPPLEMENTARY DATA

Supplementary Figure S1. Many BrdU+ beta cell nuclei co-label with pHH3, suggesting progression of BrdU-labeled cells to the mitotic phase of the cell cycle. Mouse islet cells were dispersed and cultured in 15mM glucose for 72 hours, with BrdU present for the final 24 hours, then fixed and stained for insulin, BrdU, pHH3 and dapi. Many BrdU-labeled beta cell nuclei co-stain for pHH3 (arrows). Given the long duration of BrdU exposure, some of the BrdU(+) pHH3(-) cells may have progressed through the cell cycle entirely; note the occasional BrdU doublets, which are negative for pHH3.



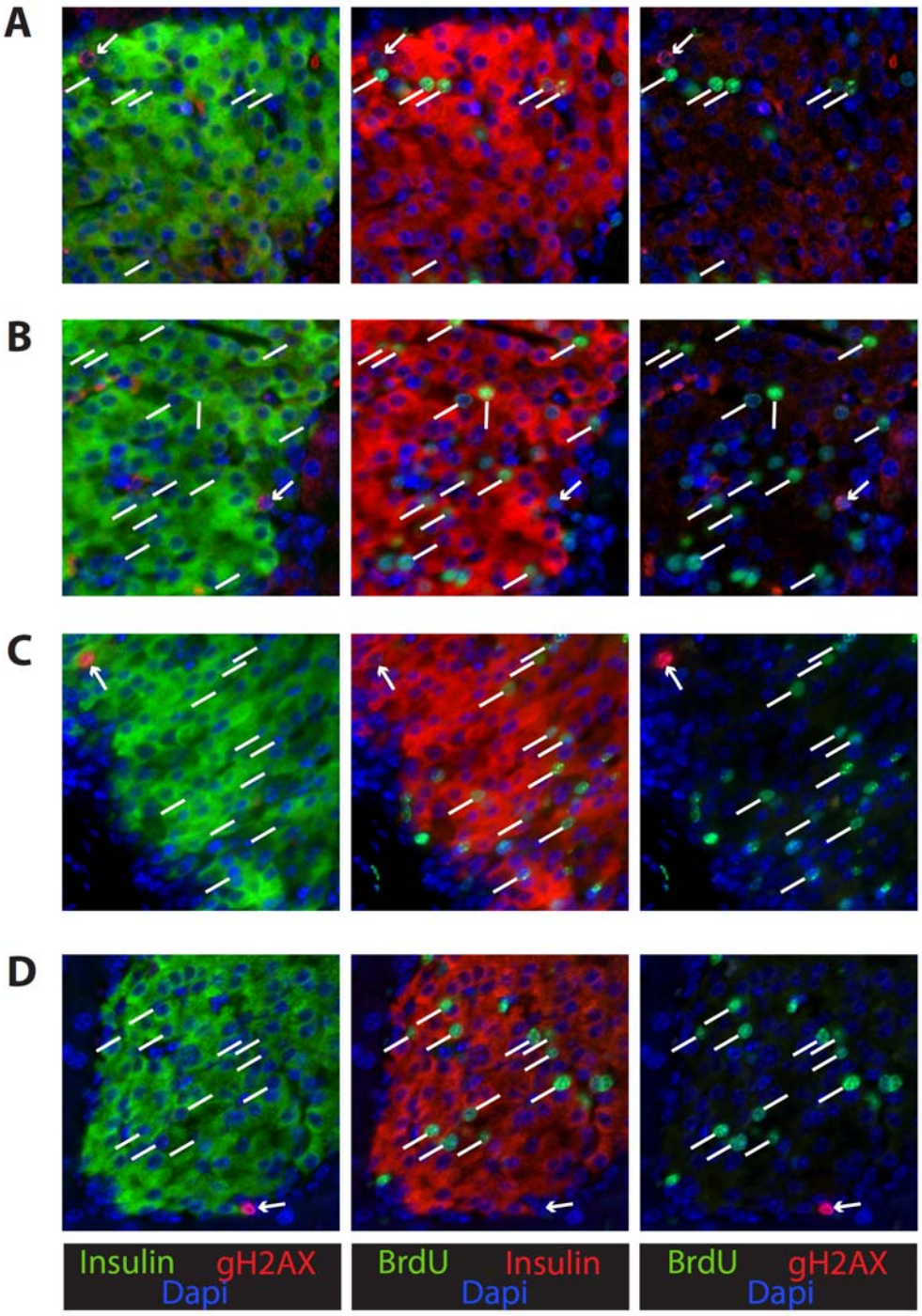
SUPPLEMENTARY DATA

Supplementary Figure S2. In vivo HFD-stimulated beta cell proliferative expansion leads to very few gH2AX-labeled beta cell nuclei. Pancreas sections from control diet (A) or high fat diet (HFD; B-C) fed mice were stained for insulin, BrdU, gH2AX and dapi. All gH2AX-stained islet nuclei identified in this experiment are shown above; the vast majority of islets imaged contained no detectable gH2AX-labeled cells. Although many insulin+ cells labeling with BrdU were found (white lines), only four gH2AX+ nuclei (white arrows) were found in the 8 sections. The gH2AX+ nucleus in (A) appears to be a non-insulin-positive cell, whereas the gH2AX+ nuclei in (B-C) appear to belong to insulin+ cells. The gH2AX+ nuclei in (B) are also BrdU+, but those in (A) and (C) are BrdU-negative.



SUPPLEMENTARY DATA

Supplementary Figure S3. In vivo hyperglycemia-stimulated beta cell proliferative expansion leads to very few gH2AX-labeled beta cell nuclei. Pancreas sections from mice rendered continuously hyperglycemic for 4 days by intravenous glucose infusion were stained for insulin, BrdU, gH2AX and dapi. As with the HFD experiment shown in Suppl. Fig 2, all gH2AX-stained islet nuclei identified in this experiment are shown above. Again, the vast majority of islets imaged contained no detectable gH2AX-labeled cells. Although many insulin+ cells labeling with BrdU were found (white lines), only four gH2AX+ nuclei (white arrows) were found in pancreas sections from four mice. The gH2AX+ nucleus in (D) appears to be a non-insulin-positive cell, whereas the gH2AX+ nuclei in (A-C) appear to belong to insulin+ cells. In this hyperglycemia experiment, none of the gH2AX+ nuclei were also BrdU+.



SUPPLEMENTARY DATA

Supplementary Figure S4. Ex vivo cultured intact islets labeled infrequently for gH2AX unless exposed to DNA damaging agent Mitomycin C, and very rarely labeled for both BrdU and gH2AX. Whole mouse islets were cultured for 72 hours in islet medium containing 5mM glucose, 15mM glucose or 15mM glucose with Mitomycin C, with BrdU added for the final 24 hours of culture. The islets were then fixed, embedded in paraffin, sectioned and stained for insulin, BrdU, gH2AX and dapi (A). Some insulin+ cells labeled with BrdU (white lines, B) or gH2AX (white arrows, C) but very few of the gH2AX+ nuclei were also BrdU+ (D).