Sirolimus induces depletion of intracellular calcium stores and mitochondrial dysfunction in pancreatic beta cells

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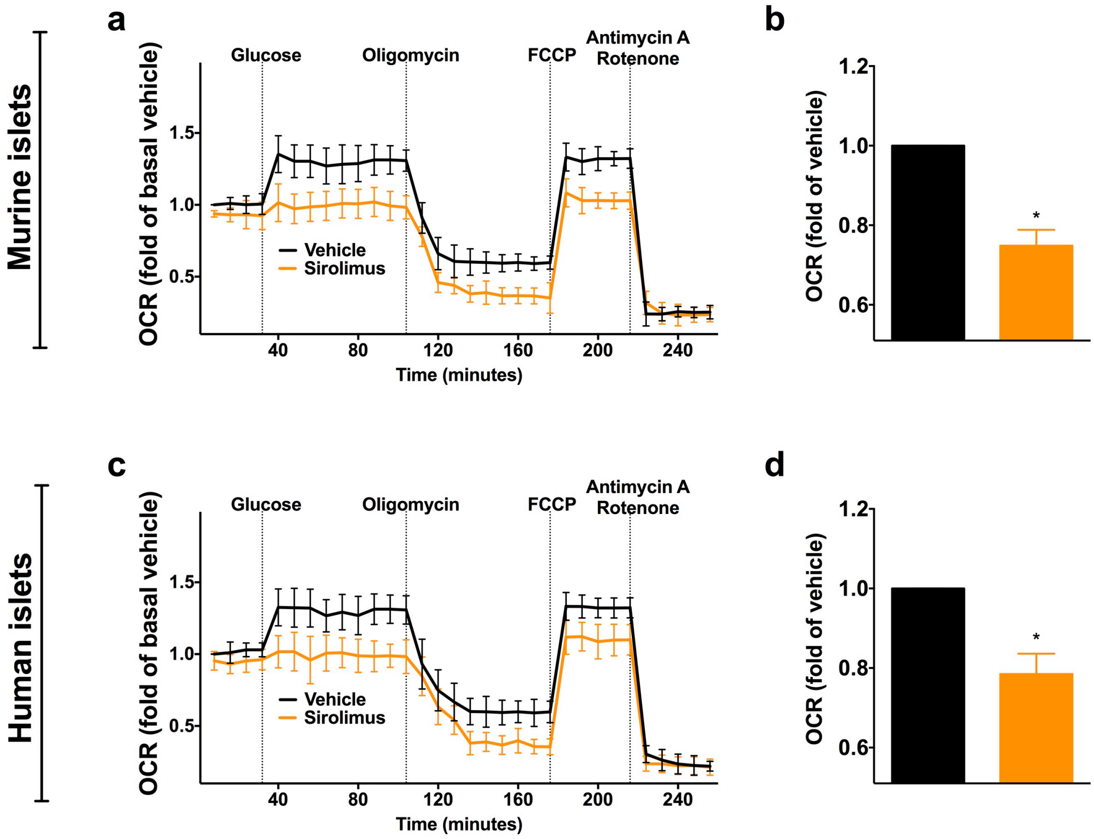
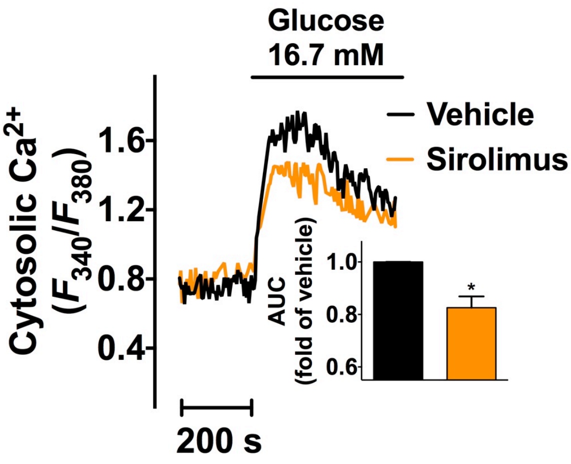
Supplementary Figures



Supplementary Figure 1.

Sirolimus impairs mitochondrial respiration in murine and human islets.

Oxygen consumption rate (OCR) was measured using the Extracellular Flux Analyzer in murine (**a,b**) and human (**c,d**) islets incubated for 24h with vehicle or 25 nM sirolimus and then treated with glucose, oligomycin, phenylhydrazone (FCCP), antimycin A, and rotenone. The maximal respiratory capacity is quantified in panels **b** and **d**. Data are presented as mean ± s.e.m. of 3 independent experiments. *:p<0.05 *vs* vehicle.



Supplementary Figure 2.

Effects of sirolimus on cytosolic Ca^{2+} dynamics in clonal β cells.

Representative curves of cytosolic Ca^{2+} dynamics in INS-1 β cells loaded with Fura-2 AM in response to glucose 16.7 mM. **Inset**: Area under curve (AUC); data are presented as mean±s.e.m of 5 independent experiments performed in triplicate. *:p<0.05 *vs* vehicle.

Supplementary Table 1– Characteristics of human islet donors

Donor	Age	Sex	BMI (kg/m^2)
1	27	M	30
2	61	M	27
3	30	M	25.9
4	45	M	36.4

BMI: Body mass index.

Supplementary Table 2 – Sequences of oligonucleotide primers and product sizes (in bp)

	Human	Mouse	Rat
IP3R1	GGGTTCAAGAGGGAATGGGA	CGGCTGCTCTCTCATTTGTC	ACCTTCTCCTCCCATTGGTG
	CCATGTCCCCACTTCTCTGT	CTTGGTTAGCTGAGGTGGGA	CATCCGAAGAGTGCACATGG
	100	95	90
IP3R2	ACCTGGATTGCTGTGCTAGT	TCTCAGCTGTCCTCTCCTCT	CAGCGAATCTCTTCCGTGTG
	AAAAGATCCCTCTGGCTGCT	AGAGCAACTGGGAGGAGAAC	TGCATTGTGGGTAGGGTCAT
	102	107	103
IP3R3	GAGAAGTCAGAGCTGTGGGT	CTACACAGACATGCGGCTTC	AGGAATGGGTCTTCTGTGGG
	GGAGCCCCACTTACCTCTTT	TCTGACTTCCCCGTGGTAAC	ACTTTCTCCCTGCCTCTTCC
	98	110	111
RyR2	CCTTGCCTGAGTGCAGTTG	TTCCCCAAGATGGTGACAAG	ACTTATGAGCACAGCAGGGT
	TTGAGGTATCAACAGGTTGTGG	CTCCAGCAGGTAGCTCAGGT	AGGGAATTTGGGGAGGAAGG
	130	105	100
SERCA2b	TCGTTATGTTCAGCCCAGGT	CATGTCTAGCAGAACACGGC	AATTGTCGTAGGTGCATCGC
	CATGCCTGTAATCCCAGCAC	CACCACACTTAACCTGGCAC	AGCCAGCAGAGACAGGAAAT
	91	96	98
SERCA3	AAGGAAGGAGGCAGCATCTT	AGACAGACCTGACCTTCGTG	GCTTGTGTTCCCGTTTCTGT
	TGAAGGACCCAATTCTCCCC	CATGACCACTCGAATGCCAG	GGCCCAAAAGTGAGAAGGTG
	111	107	103
GAPDH	GGGACTGGCTTTCCCATAAT	CACTGAGCATCTCCCTCACA	TCTCCCTCACAATTCCATCC
	TGTGGTCTGCAAAAGGAGTG	GTGGGTGCAGCGAACTTTAT	AGAGGGTGCAGCGAACTTTA
	94	111	99

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