SUPPLEMENTAL INFORMATION:

MATERIALS AND METHODS:

Cell culture and Ad transduction: Cell lines, HKC8, a transformed human kidney proximal tubule cell line with high PTH1R expression, 293, a human embryonic kidney cell line which lacks PTH1R expression, and 293 stably transfected with human PTH1R cDNA were grown in DMEM medium supplemented with 10% FCS and 1% penicillin/streptomycin.

Ad constructs with cDNAs were made as described previously and their concentration determined by optical density at 260nm and by plaque assay (8,19). Specific cDNAs used were human cyclin E and cdk2, human PTHrP cDNA, wild-type or the SP deletion mutant, with hemagglutinin (HA) tag at their C-terminus ends. Ad-beta-galactosidase (LacZ), Ad-Cre recombinase (Cre) or Ad-green fluorescent protein (GFP), were used as controls.

For adenoviral transduction of whole islets, 400-2000 islet equivalents (IEQs; 1 IEQ=125µm diameter) were hand-picked under the microscope, and incubated for 1h in a 3cm non-tissue culture petri-dish at 37°C in 250µl of islet media (RPMI supplemented with 5.5mM glucose, 10% FCS and 1% penicillin/streptomycin) without serum, together with adenovirus added at a MOI of 500, assuming 1000 cells/IEQ. Subsequently 2 ml of complete islet media was added and the incubation was continued for 24h for the insulin content and GSIS experiments (Fig 2D, E), 48h for the quantitation of PTHrP and PTH1R (Fig 1), and 72h for the cell cycle analysis (Fig 4A-C). Human islet cell cultures used for proliferation studies were made and transduced with Ad constructs at 100 MOI as described in detail (8). Briefly, cells were transduced with the Ad constructs at the time of trypsinization, and kept in complete islet media for 24h. Subsequently, they were cultured overnight in serum-free islet media, and then treated with 1 µl/ml BrdU (Amersham, Pharmacia Biotech, Piscataway, NJ) in serum-free media for an additional 24h, after which they were fixed in 2% paraformaldehyde for 30 min at room temperature.

For peptide treatment, whole islets or islet cell cultures were kept in serum-free medium overnight and subsequently treated with either vehicle (10mM acetic acid) or PTHrP peptides (1-36) or (38-94) at 100nM for an additional 24h also in serum-free medium. For the proliferation experiments, BrdU was added to the serum-free medium for the last 24h with the peptide, and cells were subsequently fixed. The dose of peptide used was based on our previous studies in rodent β-cells (8). PTHrP(1-36) was synthesized by solid phase synthesis, purity and bioactivity measured as described previously (15,16,Suppl ref 1).

Proliferation: To analyze cell cycle progression, whole human islets were transduced with Ad constructs, dispersed 72 hrs after transduction into single cells with 0.05% trypsin-EDTA (Invitrogen, Carlsbad, CA), suspended in chilled 70% ethanol, and kept overnight at 4°C. Fixed cells were treated with 100 units/ml RNAase A (Sigma) in PBS and stained with 50µg/ml propidium iodide (Sigma) at room temperature for 30min after which flow cytometric analysis was performed.

β-cell proliferation in human islet cell cultures was quantitated on an average of 11,700 (Figure 2) and 4,300 (Figure 4) insulin-positive cells / condition/ human islet prep. Analysis of transduction rates in primary human islet cells was performed by HA and insulin staining (8). Magnification of photomicrographs is 200X, except for Fig 2A which is a 600X confocal image.

Gene expression: For real time PCR, RNA was extracted using the RNeasy micro kit (Qiagen, Valencia, CA). 0.5-1µg of total RNA was reverse transcribed in a total volume of 20µl, and then diluted to 100µl with 2µl of the total reaction used as input for PCR using SYBR Green Master Mix (Applied Biosystems, Foster City, CA), and analyzed on an ABI 7300 Real Time PCR System. Primers used are listed in Supplemental Table 1.

The primary antibodies used for western blot analysis were against PTH1R (Babco, Richmond, CA), PTHrP, tubulin (EMD Chemicals, Gibbstown, NJ), actin (Sigma, St. Louis, MO), cyclin D1 (Neomarkers, Freemont, CA), p27 (BD Pharmingen, San Diego, CA), cdk6 (Abcam, Cambridge, MA), cyclin D3, HA, (Cell Signaling, Danvers, MA) cyclin E, cdk4, p16, p18, p21, and cdk2 (Santa Cruz Biotechnology, Santa Cruz, CA).

PTHrP in the culture medium (Fig 1) was quantitated using a modification of a previously described human PTHrP(1-74) immunoradiometric assay (Suppl ref 1). The modified assay measures PTHrP(1-36). The detection limit of this assay is 2pM. Quantification of PTHrP(1-36) remaining in the culture medium at the end of the proliferation experiments in Fig 2 was performed by an enzyme immunoassay (Peninsula Laboratories Inc, San Carlos, CA) according to the manufacturer's protocol.

REFERENCES:

1) Burtis WJ, Brady TG, Orloff JJ, Ersbak JB, Warrell RP Jr, Olson BR, Wu TL, Mitnick ME, Broadus AE, Stewart AF. Immunochemical characterization of circulating parathyroid hormone-related protein in patients with humoral hypercalcemia of cancer. N Engl J Med. 1990;322:1106-1112

Supplemental Table 1: List and sequence of primers used.

1	cyclinD1 (Forward)	CAGGCGGCTCTTTTTCAC
	cyclinD1 (Reverse)	CCCTCGGTGTCCTACTTCAA
2	cyclinD2 (Forward)	CAGGAACATGCAGACAGCAC
	cyclinD2 (Reverse)	GGACATCCAACCCTACAT
3	cyclinD3 (Forward)	GCACAGTTTTTCGATGGTCA
	cyclinD3 (Reverse)	TGGATGCTGGAGGTATGTGA
4	Cdk4 (Forward)	AAAGCCACCTCACGAACTGT
	Cdk4 (Reverse)	TGAGGGTCTCCCTTGATCTG
5	Cdk6 (Forward)	GAAAGTCCAGACCTCGGAGA
	Cdk6 (Reverse)	CGTGGTCAGGTTGTTTGATG
6	Cyclin E1 (Forward)	TACACCAGCCACCTCCAGACAC
	Cyclin E1 (Reverse)	CCTCCACAGCTTCAAGCTTTTG
7	Cyclin E2 (Forward)	CCCAAGAAGCCCAGATAATCC
	Cyclin E2 (Reverse)	AATACAGGTGGCCAACAATTCC
8	Cyclin A1 (Forward)	AGCACCCTGCTCGTCACTTG
	Cyclin A1 (Reverse)	CGGTCCTTCCCAGCTGAGATAC
9	Cyclin A2 (Forward)	TGCTGGAGCTGCCTTTCATT
	Cyclin A2 (Reverse)	TGAAGGTCCATGAGACAAGGCT
10	Cdk1 (Forward)	ACCAGGAAGCCTAGCATCCC
	Cdk1 (Reverse)	GATTCAGTGCCATTTTGCCAG
11	cdk2 (Forward)	TGGATGCCTCTGCTCTCACTG
	cdk2 (Reverse)	GAGGACCCGATGAGAATGGC
12	P21 (Forward)	CTGCCTCCTCCCAACTCAT
	P21 (Reverse)	GCGACTGTGATGCGCTAAT
13	p27 (Forward)	CGAGCTGTTTACGTTTGACG
	P27 (Reverse)	CATTTGGTGGACCCAAAGAC
14	p57 (Forward)	GCGGCGATCAAGAAGCTGTC
	p57 (Reverse)	CCGGTTGCTGCTACATGAAC
15	p16 (Forward)	ATGGAGCCTTCGGCTGACTGGCTG
	p16 (Reverse)	CGAGGTTTCTCAGAGCCTCTCTGG
16	p18 (Forward)	ATGGATTTGGAAGGACTGCG
	p18 (Reverse)	ATGACAGCGAAACCAGTTCG
17	P19 (Forward)	TGCAAAGGATCCACCAGGGTCTGA
	P19 (Reverse)	TGAGTGGGATCCTTGAGCTGCTGC
18	Insulin (Forward)	TCACACCTGGTGGAAGCTCTCTA
	Insulin (Reverse)	ACAATGCCACGCTTCTGCAGGGAC
19	Glucagon (Forward)	GATGAACGAGGACAAGCGCC
	Glucagon (Reverse)	TCACCAGCCAAGCAATGAAT
20	Somatostatin (Forward)	CCCAGACTCCGTCAGTTTCT
	Somatostatin (Reverse)	ATCATTCTCCGTCTGGTTGG
21	Glut 2 (Forward)	GCTACCGACAGCCTATTCTA
	Glut 2 (Reverse)	CAAGTCCCACTGACATGAAG
22	Glucokinase (Forward)	CTTCCCTCAGTTTTTCGGTGG
	Glucokinase (Reverse)	TTGATTCCAGCGAGAAAGGTG
23	Pdx1 (Forward)	ACCAAAGCTCACGCGTGGAAA
	Pdx1 (Reverse)	TGATGTGTCTCTCGGTCAAGTT
24	Maf A (Forward)	CTTCAGCAAGGAGGAGGTCATC
	Maf A (Reverse)	CTCGTATTTCTCCTTGTACAGGTCC
25	Maf B (Forward)	TGAACTTTGCGCGTTAAGCC
	Maf B (Reverse)	CACGCAGCCGCCGGAGTTTC
26	Nkx6.1 (Forward)	ACACGAGACCCACTTTTTCCG
	Nkx6.1 (Reverse)	TGCTGGACTTGTGCTTCTTCAAC
27	Nkx2.2 (Forward)	TGACTCTCGGCTCCACTAGG
	Nkx2.2 (Reverse)	CGGCTGACAATATCGCTACTCA
28	NeuroD1 (Forward)	GACTGAACGCGGCGCTAGAC
	NeuroD1 (Reverse)	CGGCGGAGGCTTAACGTGGA
29	Isl1 (Forward)	ATTTCCCTATGTGTTGGTTGCG
	Isl1 (Reverse)	CGTTCTTGCTGAAGCCGATG
30	PTH1R (Forward)	GCACTGCACGCGCAACTA
	PTH1R (Reverse)	GCTCACGGCGCGCA
31	Actin (Forward)	CATGTACGTTGCTATCCAGGC
	Actin (Reverse)	CTCCTTAATGTCACGCACGAT