**LETTER**

IAPP–driven metabolic reprogramming induces regression of p53–deficient tumours in vivo

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*TP53* is commonly altered in human cancer, and *Tp53* reactivation suppresses tumours in vivo in mice—(TP53 and Tp53 are also known as *p53*). This strategy has proven difficult to implement therapeutically, and here we examine an alternative strategy by manipulating the p53 family members, *Tp53* and *Tp73* (also known as *p63* and *p73*, respectively). The acidic transactivation-domain-bearing (TA) isoforms of p63 and p73 structurally and functionally resemble p53, whereas the AN isoforms (lacking the acidic transactivation domain) of p63 and p73 are frequently overexpressed in cancer and act primarily in a dominant-negative fashion against p53. TAp63 and TAp73 to inhibit their tumour-suppressive functions.1–4. The p53 family interacts extensively in cellular processes that promote tumour suppression, such as apoptosis and autophagy1–4, thus a clear understanding of this interplay in cancer is needed to treat tumours with alterations in the p53 pathway. Here we show that deletion of the AN isoforms of p63 or p73 leads to metabolic reprogramming and regression of p53–deficient tumours through upregulation of IAPP, the gene that encodes amylin, a 37–amino-acid peptide co-secreted with insulin by the β cells of the pancreas. We found that IAPP is causally involved in this tumour regression and that amylin functions through the calcitonin receptor (CalcR) and receptor activity modifying protein 3 (RAMP3) to inhibit glycolysis and induce reactive oxygen species and apoptosis. Pramlintide, a synthetic analogue of amylin that is currently used to treat type 1 and type 2 diabetes, caused rapid tumour regression and senescence (Extended Data Fig. 2f–j), and apoptosis (Extended Data Fig. 2k–o). We also examined changes in lifespan (Extended Data Fig. 2a–c) and suggesting that the presence of the stromal cells (Extended Data Fig. 4v). These data indicate that inhibition of the AN isoforms of p63 and p73 serves to upregulate TAp63 and TAp73 to compensate for loss of p53 in tumour suppression. Indeed, TAp63 and TAp73 are higher in ANp63+/− or ANp73+/− and ANp73+/− mice (Extended Data Fig. 3a–c) with an increase in apoptosis (Extended Data Fig. 3d–h) and senescence (Extended Data Fig. 3i–m).

To determine whether TAp63 or TAp73 compensate for p53 function in tumours in vivo, we acutely removed ANp63 or ANp73 by intratumoral injection with adenovirus–Cre-mCherry (Extended Data Fig. 4a–d and Fig. 1a–f) in ANp63+/− or ANp73+/− and ANp73+/− at 10 weeks of age. Tumours were 2.3–5.8 mm3 in size at the time of infection and monitored weekly by magnetic resonance imaging (MRI, Fig. 1a–i). Mice deficient for either ANp63 or ANp73 and p53 showed marked decreases in tumour burden (Fig. 1h, i). The reduction of ANp63 and ANp73 expression resulted in increased expression of TAp63 and TAp73 (Fig. 1j–m and Extended Data 4d) and increased apoptosis (Extended Data Fig. 4e–h) and senescence (Extended Data Fig. 4i–k). ANp63+/− or ANp73+/− mice also had an increased lifespan (Fig. 1n). We found differences in CD4/CD8–positive cells in young mice (4 weeks) (Extended Data Fig. 4l–p), indicating that changes in T-cell development may lead to a lower tumour incidence in double-mutant mice. Indeed, we found that p53+/− thymic lymphomas are composed primarily of CD4/CD8 double-positive thymocytes while the ANp63+/− or ANp73+/− and ANp73+/− lymphomas contain very few CD4/CD8 double-positive thymocytes (Extended Data Fig. 4q–t). Lastly, we asked whether thymic stromal cells contribute to the apoptosis in the regressing lymphomas. We sorted CD45-positive cells to select for T lymphocytes in T lymphomas from mice deficient for ANp63 or ANp73 or p53, and ANp63 or ANp73 and p53 plus p53+/− thymocytes underwent apoptosis independent of the presence of the stromal cells (Extended Data Fig. 4v). These data indicate that inhibition of the AN isoforms of p63 and p73 bind to the promoters of the TA isoforms of p63 and p73, suggesting that the AN isoforms of p63 and p73 can transcriptionally repress TAp63 and TAp73 transcription (Extended Data Fig. 5a–g). We also found that the increase in apoptosis and cellular senescence was dependent on TAp63 and TAp73 (Extended Data Fig. 5j–q).

We performed RNA sequencing of lymphomas after infection with Ad-mCherry (ANp63+/− or ANp73+/− or Ad-Cre-mCherry (ANp63+/− or ANp73+/−) and Ad-Cre-mCherry (ANp63+/− or ANp73+/−)) and found that thymic lymphomas from mice deficient for p53 and ANp63 clustered with those from mice deficient for p53 and ANp73 (Extended Data Fig. 6a). Ingenuity pathway analysis (IPA) (Fig. 1q) revealed genes involved in

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metabolism including TP53-inducible glycolysis and apoptosis regulator (TIGAR)\(^{17}\), and glutaminase 2 (GLS2)\(^{20,21}\). While we found that TIGAR and GLS2 were upregulated in either A\(\Delta Np63^{+/+};p53^{−/−}\) or A\(\Delta Np73^{+/-};p53^{−/−}\) thymic lymphomas, we identified a novel gene, islet amyloid polypeptide (IAPP) or amylin, which was upregulated by over fivefold in both double-mutant thymic lymphomas. IAPP limits glucose uptake, resulting in increased intracellular glucose-6-phosphate (G-6-P)\(^{23}\) levels and decreased glycolysis\(^{24}\). We validated IAPP, TIGAR and GLS2 expression in thymic lymphomas derived from A\(\Delta Np63^{+/-};p53^{−/−}\) and A\(\Delta Np73^{+/-};p53^{−/−}\) mice and found that IAPP is expressed at levels over twofold higher in double-mutant mice (Fig. 1p and Extended Data Fig. 6b–d). IAPP and GLS2 expression depend on TAP63 and TAP73 (Fig. 1q and Extended Data Fig. 6d). To determine whether TAP63 or TAP73 transcriptionally regulate IAPP, we performed chromatin immunoprecipitation in mouse embryonic fibroblasts (MEFs; Extended Data Fig. 6e–g) and thymocytes (Fig. 1r,s). We found that TAP63 and TAP73 bind to sites located in the promoter (site 1), 1,756 nucleotides upstream of the transcriptional start site, and intron 2 (site 2) of IAPP, 706 nucleotides downstream of the transcriptional start site (Extended Data Fig. 6e–g). Because a greater binding affinity of TAP63 and TAP73 was detected in the promoter region (site 1) of IAPP, we cloned this site into a luciferase reporter gene and also mutated this site (Extended Data Fig. 6h–k). Only the luciferase reporter gene containing wild-type IAPP promoter site 1 was transactivated by TAP63 and TAP73 whereas the mutant version was not. Taken together, these data indicate that IAPP is a transcriptional target gene of TAP63 and TAP73 (Fig. 1i).

Expression of IAPP in p53\(^{−/−}\) MEFs resulted in lower levels of glycolysis compared to that in A\(\Delta Np63^{−/−};p53^{−/−}\) and A\(\Delta Np73^{−/−};p53^{−/−}\) MEFs (Extended Data Fig. 6l–m and Fig. 1u). Conversely, when we knocked down IAPP in A\(\Delta Np63^{−/−};p53^{−/−}\) and A\(\Delta Np73^{−/−};p53^{−/−}\) MEFs, the levels of glycolysis were similar to that of p53\(^{−/−}\) MEFs (Fig. 1u) indicating that IAPP inhibits glycolysis. In vivo, we detected massive tumour regression in A\(\Delta Np63^{−/−};p53^{−/−}\) or A\(\Delta Np73^{−/−};p53^{−/−}\) thymic lymphomas treated with Ad-shIAPP-mCherry (Extended Data Fig. 7a, b). Additionally, A\(\Delta Np63^{−/−};p53^{−/−}\) or A\(\Delta Np73^{−/−};p53^{−/−}\) thymic lymphomas treated with Ad-shIAPP-mCherry show increased thymic lymphoma-free survival compared to thymic lymphoma-free survival period compared to p53\(^{−/−}\) mice or A\(\Delta Np63^{−/−};p53^{−/−}\) and A\(\Delta Np73^{−/−};p53^{−/−}\) mice treated intratumorally with Ad-shIAPP-mCherry (Extended Data Fig. 7a, b), indicating that IAPP is a tumour suppressor gene and is causally involved in the in vivo effects seen upon inactivation of A\(\Delta Np63\) or A\(\Delta Np73\). Given that pramlintide, a synthetic analogue of amylin, is used to treat type I and type II diabetes\(^{22}\), we treated thymic lymphomas in A\(\Delta Np63^{−/−};p53^{−/−}\) and A\(\Delta Np73^{−/−};p53^{−/−}\) mice. Indeed, three-week intratumoral injections resulted in rapid tumour regression (Fig. 2e,l,s), P < 0.005 at 13 weeks. This effect was exacerbated by systemic intravenous treatment with thymic lymphomas 48 h after infection with adenoviruses. Red oval indicates significant upregulated metabolic genes, p, q, qRT–PCR for IAPP in thymic lymphomas (p) or MEFs of the indicated genotypes using a non-targeting shRNA (shNT) or shRNAs for TAP63 (shTAP63) or TAP73 (shTAP73) (q), n = 4, P < 0.005. r, s, qRT–PCR of IAPP promoter site 1 using chromatin immunoprecipitation, n = 3, P < 0.005. t, Cartoon showing transcriptional activation of IAPP by TAP63 and TAP73. u, Extracellular acidification rate (ECAR) as a measurement of glycolysis, P < 0.005.
ensue and may be at play in the thymic lymphoma cells that acutely by acquiring additional mutations and compensatory mechanisms often the accumulation of ROS. Additionally, cancer cells tightly regulate ROS by inhibition of glycolysis, nutrient deprivation or excess can result in inhibits glycolysis similarly to 2DG and leads to oxidative stress that in glycolysis in as a proxy of glycolysis within the tumours, we found a marked reduction inactivation of p53-deficient thymic lymphomas. a

**Figure 2** | IAPP is causally involved in tumorigenesis suppression in p53-deficient thymic lymphomas. a–n, Thymic lymphomas were infected with adenovirus (Ad)-mCherry (a, h), Ad-IAPP-mCherry (+IAPP) (b, i), Ad-shIAPP-mCherry (c, d, j, k), or treated with pramlintide intratumorally (IT) (e, l) or intravenously (IV) (f, m), or with 2DG (g, n). Yellow dashed lines indicate tumour. Volume of tumour shown. UN-D, undetectable.

Pramlintide (2f, m, t and Extended Data Fig. 7c–q), P < 0.005, similar to that seen in tumours treated with a known inhibitor of glycolysis, 2-deoxy-D-glucose (2DG; Fig. 2g, n, u). These data provide preclinical in vivo evidence that pramlintide can be used to effectively treat p53-deficient tumours. Using in vivo dynamic magnetic resonance spectroscopy to measure the conversion of hyperpolarized [1-13C]pyruvate to lactate as a proxy of glycolysis within the tumours, we found a marked reduction in glycolysis in ΔNp63ΔNp73 and ΔNp63ΔNp73 double-deficient mice and after introducing IAPP into p53−/− thymic lymphomas similar to tumours treated with 2DG (Fig. 2v). ΔNp63ΔNp73 Δp53−/− and ΔNp73Δp53−/− thymic lymphomas infected with a short hairpin RNA for IAPP exhibited levels of glycolysis similar to those found in p53−/− thymic lymphomas (Fig. 2v). Pramlintide also inhibits glycolysis in tumours (Fig. 2v).

IAPP has been shown to induce reactive oxygen species (ROS) and activate apoptosis. We found a marked increase in the levels of ROS and apoptosis in thymic lymphomas expressing IAPP or treated with pramlintide or 2DG, whereas neither ROS nor apoptosis occurred upon inactivation of IAPP in thymic lymphomas from ΔNp63ΔNp73Δp53−/− and ΔNp73Δp53−/− mice (Fig. 2w), indicating that upregulation of IAPP inhibits glycolysis similarly to 2DG and leads to oxidative stress that triggers apoptosis. While high levels of ROS are not commonly triggered by inhibition of glycolysis, nutrient deprivation or excess can result in the accumulation of ROS. Additionally, cancer cells tightly regulate ROS by acquiring additional mutations and compensatory mechanisms often ensue and may be at play in the thymic lymphoma cells that acutely downregulate glycolysis by IAPP/p53.

To extend our findings to human cancer where p53 is altered in the majority of cases, we analysed human cancer cell lines containing p53 deletions or mutations. We used short interfering RNA (siRNA) to knockdown ΔNp63 or ΔNp73 in cells derived from a lung adenocarcinoma (H1299) (Fig. 3a). Downregulation of ΔNp63 or ΔNp73 resulted in upregulation of TAPl3, Tap73 and IAPP (Fig. 3a) and an increase in apoptosis and decrease in cell proliferation (Fig. 3b and Extended Data Fig. 8a–d). To ask whether IAPP can also inhibit glycolysis in human cancer cell lines, we transfected H1299 cells with siΔNp63, siΔNp73 or IAPP (Fig. 3a). Knockdown of ΔNp63 or ΔNp73 or expression of IAPP resulted in an inhibition of glycolysis (Fig. 3c, d) and glucose uptake (Extended Data Fig. 8e, g), accumulation of ROS (Fig. 3d–f), and induction of apoptosis (Fig. 3d, g, h). We inhibited ROS in these cells using N-acetyl-l-cysteine (NAC) and observed no apoptosis (Fig. 3d–h). Previous studies have indicated that IAPP inhibits glycolysis by increasing intracellular G-6-P in turn leading to an inhibition of hexokinase. We measured the levels of intracellular G-6-P in H1299 cells and found that cells expressing high levels of IAPP (H1299-siΔNp63, H1299-siΔNp73, or H1299 + IAPP) also had high levels of G-6-P while knockdown of IAPP resulted in a diminution in G-6-P (Extended Data Fig. 8f, g). Overexpression of glucose hexokinase II (HKII) led to a rescue of the glycolytic capacity of H1299 cells expressing siΔNp63 or siΔNp73 to levels similar to those in parental H1299 cells (Fig. 3c–g). These results indicate that IAPP inhibits glycolysis through the inhibition of HKII. We found that treatment of H1299 cells with pramlintide led to similar effects on glycolysis and apoptosis (Fig. 3g–n). Taken together, these
Figure 3 | IAPP inhibits glycolysis and induces ROS and apoptosis in p53-deficient human cell lines. a, Representative western blot analysis, n = 4. b, Immunofluorescence for apoptosis and 5′-ethynyl-2′-deoxyuridine (EdU) incorporation. c, Extracellular acidification rate as a measure of glycolysis, n = 3, P < 0.005. Legend in d is colour-coded and corresponds to panels e, f. h, Immunofluorescence and quantification for ROS (red) (e, f) or apoptosis (green or green/red) (g, h).

IAPP is a secreted protein and binds to the calcitonin receptor (CALCR) and RAMP3 (ref. 27). To determine whether IAPP functions through these receptors to inhibit glycolysis, secreted media from H1299 cells expressing siΔNp63 (siΔNp6338) or siΔNp73 (siΔNp7339), which contains secreted IAPP (Fig. 4a and Extended Data Fig. 9a, b), was added to H1299 cells resulting in inhibition of glycolysis (Fig. 4b) and induction of ROS and apoptosis (Fig. 4c, d). In contrast, when these media were used to treat H1299 cells with knockdown of CALCR or RAMP3, glycolysis was not inhibited and ROS and apoptosis were not induced (Fig. 4b–d), indicating that the CALCR and RAMP3 receptors are critical for IAPP function. We also treated the H1299 cells with media from H1299 cells expressing siΔNp63 (siΔNp6338) or siΔNp73 (siΔNp7339) and an amylin inhibitor, which led to high levels of glycolysis (Extended Data Fig. 9c) and low levels of ROS and apoptosis (Fig. 4c, d). IAPP causes activation of the NLRP3 inflammasome34, which has been shown to be anti-tumorigenic in certain cancers via IL-18 processing39. We blocked caspase-1 using an inhibitor and found that it prevented apoptosis of H1299 cells (Fig. 4d), demonstrating that pyroptosis may also be an important mechanism of action of IAPP.

To demonstrate the importance of the calcitonin receptor in vivo, we treated p53−/− mice with thymic lymphomas at 10 weeks of age with pramlintide and a calcitonin receptor inhibitor (Fig. 4e–m) and found that this inhibition rendered pramlintide ineffective, demonstrating the importance of the calcitonin receptor for IAPP/amylin/pramlintide function (Fig. 4n). To further determine the anti-tumorigenic efficacy of pramlintide and its mechanism of action, we treated H1299 cells with knockdown of CALCR or RAMP3, glycolysis was not inhibited and ROS and apoptosis were not induced (Fig. 4b–d), indicating that the CALCR and RAMP3 receptors are critical for IAPP function. We also treated the H1299 cells with media from H1299 cells expressing siΔNp63 (siΔNp6338) or siΔNp73 (siΔNp7339) and an amylin inhibitor, which led to high levels of glycolysis (Extended Data Fig. 9c) and low levels of ROS and apoptosis (Fig. 4c, d). IAPP causes activation of the NLRP3 inflammasome34, which has been shown to be anti-tumorigenic in certain cancers via IL-18 processing39. We blocked caspase-1 using an inhibitor and found that it prevented apoptosis of H1299 cells (Fig. 4d), demonstrating that pyroptosis may also be an important mechanism of action of IAPP.

Figure 4 | Calcitonin and RAMP3 receptors are required for secreted IAPP to suppress tumorigenesis. a, Cartoon depicting treatment of cells expressing siRNAs and treated with media from the cells secreting IAPP on the left. b, Extracellular acidification rate (ECAR) in H1299 cells. c, d, Immunofluorescence for ROS (c) and apoptosis (d). e–m, MRI and quantification of thymic lymphomas treated with placebo (e, h, k), pramlintide (f, i, l), or pramlintide plus calcitonin inhibitor (CalR I) (g, j, m), n = 5 mice. n, Cartoon of IAPP signalling through RAMP3 and calcitonin receptor (CALCR) to inhibit glycolysis and induce ROS and apoptosis. o, Kaplan–Meier curves from patients with p53 mutant tumours and co-expression of IAPP, RAMP3 and CALCR. Boxed numbers represent median survival.
pramlintide in cells with p53 deletions or mutations, we treated additional human cancer cell lines with pramlintide and a calcitonin receptor inhibitor, resulting in increased glycolysis, decreased ROS and apoptosis (Extended Data Fig. 9d–i). We assessed patient survival using data from the Cancer Genome Atlas (TCGA) of patients with p53 mutations and found that co-expression of IAPP, CALCR and RAMP3 correlated with better patient survival in basal breast cancer (Fig. 4o), colorectal cancer and lung squamous cell carcinoma (Extended Data Fig. 9j, k).

Reactivation of p53 activity in tumours results in tumour suppression.12,13 We have focused on interactions between the three p53 family members and have revealed a novel strategy to target p53-deficient and mutant cancers through amylin-based therapies like pramlintide.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 3 December 2013; accepted 30 September 2014.

Published online 17 November 2014.

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Acknowledgements We thank A. Jain, V. Pant, J. Jackson, A. Marisetty, K. Michel and the Small Animal Imaging Facility (SAIF) for technical advice. This work was supported by grants to E.R.F. from NCI (R01CA160394) and (R01CA134796), CPRIT (RP120124), NCI-Cancer Center Core Grant (CA-16672) (University of Texas M.D. Anderson Cancer Center), a development award from the Lymphoma SPORE (P50CA136411), the Hildegarde E and Olga M. Flores Foundation, and the Mel Klein Foundation and grant to J.A.B. from CPRIT (RP101243-P5). E.R.F. is a scholar of the Leukemia and Lymphoma Society, the Rita Allen Foundation and the V Foundation for Cancer Research. A.V. is a Schissler Scholar and D.C. is a CPRIT Scholar (RP101502).

Author Contributions A.V. and E.R.F. conceived the study, designed experiments and analysed data. A.V., P.R., W.N., D.C., X.S., S.K.S., M.S.R., J.L., C.V.K., E.F.S., K.N., J.P.-T., J.A.B., and K.Y.T. designed and performed experiments. P.H.G., C.C. and K.R. performed bioinformatic analyses. E.R.F. and A.V. wrote the paper. All authors discussed the paper and commented on the manuscript.

Author Information The RNA-Seq data has been deposited in the Gene Expression Omnibus (GEO) data repository and can be accessed using database accession number GSE60827. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to E.R.F. (elsaflores@mdanderson.org).
METHODS

Generation of ΔNp73 conditional knockout mice. The Cre-loxP strategy was used to generate the ΔNp73 conditional knockout allele (ΔNp73f). Genomic p73 DNA from intron 3 to intron 3 was amplified from BAC clone DNA (BAC RP23-1868N, Children’s Hospital Oakland Research Institute). loxP sites flanking exon 3 of p73 and neomycin (neo) gene flanked by flit sites inserted in intron 3 were cloned into pL253. Mouse embryonic stem cells (G4) electroporated with the targeting vector were analysed by Southern blot analysis for proper targeting of the ΔNp73 allele. Resulting chimaeras were mated with C57BL/6 albino females and genotyped as described below. Mice with germ line transmission of the targeted allele (conditional, flox neo allele, fn) were crossed to the FLP expressing mice to delete the neo cassette. Resulting progeny were intercrossed with ΔNp63-cre (C57BL/6) transgenic mice. ΔNp73fl;ΔNp63fl homozygous mice were intercrossed to generate ΔNp73−/− mice. The ΔNp73−/− mice were intercrossed to generate ΔNp73−/−;ΔNp63−/− mice. Compound mutant mice were generated by intercrossing the ΔNp63−/− and ΔNp63fl mice (ref. 15) and the ΔNp73−/− and ΔNp63fl mice with the p53−/− mice6. All procedures were approved by the IACUC at University of Texas M.D. Anderson Cancer Center.

Genotyping. Genomic DNA from tail biopsies was genotyped by Southern blot analysis by digesting genomic DNA with AluII and HindIII or by PCR using the following primers and annealing temperatures: (1) for wild type: forward, 5′-ACAGTCCTCGTCCTGTGGTTGTTA-3′ and reverse (fl-R), 5′-CCACACAGACCTCGGCCTCC-3′ (annealing temp: 58 °C); (2) for ΔNp73fl, 5′-CATGACATGGGCTGCTGT-3′ and reverse (ΔNp73fl-R): 5′-TGTCCTGCTGCTGGTTGTAT-3′, annealing temp: 63 °C; and (3) ΔNp73fl homozygous: forward: 5′-GGGGATTGGGAGAAAACGACAAATC-3′ and reverse (ΔNp73fl-R): 5′-TGTCCTGCTGCTGGTTGTAT-3′, annealing temp: 60 °C (and 4) for ΔNp73KO: ΔNp73KO forward: 5′-CTTGCAGCAAGCAGTGTTTTGTTTGC-3′ and reverse, 5′-TGTCCTGCTGCTGTTGTTAT-3′, annealing temp: 58 °C. Primers used for genotyping for the Cre gene are as follows: Cre, 5′-TGTCGCCGCCGCTGTGCAAGT-3′ and Cre-R, 5′-CGGCACCTCCAATGCCTTGGT-3′, annealing temp: 60 °C. The primers for the ΔNp63WT, ΔNp63KO, ΔNp63Box and p53 were previously described2,4,5.

Cell lines. Mouse embryonic fibroblasts (MEFs) for the indicated genotypes were generated as described previously8. Human lung adenocarcinoma cells (H1299), colorectal adenocarcinoma cells (SW-480) and breast adenocarcinoma cells (MDA-MB-231) were purchased from ATCC and cutaneous SCC cell lines (SRB12, COLO16)30 and rectal adenocarcinoma cells (H1600) were generated as described previously9. Human lung adenocarcinoma cells (H1299), colorectal adenocarcinoma cells (SW-480) and breast adenocarcinoma cells (MDA-MB-231) were purchased from ATCC and cutaneous SCC cell lines (SRB12, COLO16)30 and rectal adenocarcinoma cells (H1600) were generated as described previously9. Human lung adenocarcinoma cells (H1299), colorectal adenocarcinoma cells (SW-480) and breast adenocarcinoma cells (MDA-MB-231) were purchased from ATCC and cutaneous SCC cell lines (SRB12, COLO16)30 and rectal adenocarcinoma cells (H1600) were generated as described previously9. Human lung adenocarcinoma cells (H1299), colorectal adenocarcinoma cells (SW-480) and breast adenocarcinoma cells (MDA-MB-231) were purchased from ATCC and cutaneous SCC cell lines (SRB12, COLO16)30 and rectal adenocarcinoma cells (H1600) were generated as described previously9. Human lung adenocarcinoma cells (H1299), colorectal adenocarcinoma cells (SW-480) and breast adenocarcinoma cells (MDA-MB-231) were purchased from ATCC and cutaneous SCC cell lines (SRB12, COLO16)30 and rectal adenocarcinoma cells (H1600) were generated as described previously9. Human lung adenocarcinoma cells (H1299), colorectal adenocarcinoma cells (SW-480) and breast adenocarcinoma cells (MDA-MB-231) were purchased from ATCC and cutaneous SCC cell lines (SRB12, COLO16)30 and rectal adenocarcinoma cells (H1600) were generated as described previously9. Human lung adenocarcinoma cells (H1299), colorectal adenocarcinoma cells (SW-480) and breast adenocarcinoma cells (MDA-MB-231) were purchased from ATCC and cutaneous SCC cell lines (SRB12, COLO16)30 and rectal adenocarcinoma cells (H1600) were generated as described previously9. Human lung adenocarcinoma cells (H1299), colorectal adenocarcinoma cells (SW-480) and breast adenocarcinoma cells (MDA-MB-231) were purchased from ATCC and cutaneous SCC cell lines (SRB12, COLO16)30 and rectal adenocarcinoma cells (H1600) were generated as described previously9. Human lung adenocarcinoma cells (H1299), colorectal adenocarcinoma cells (SW-480) and breast adenocarcinoma cells (MDA-MB-231) were purchased from ATCC and cutaneous SCC cell lines (SRB12, COLO16)30 and rectal adenocarcinoma cells (H1600) were generated as described previously9.

Immunohistochemistry. Mice thymic lymphomas or thymus were dissected, fixed in 10% formalin, and embedded in paraffin. Sections were de-waxed in xylene and re-hydrated using decreasing concentrations of ethanol. Antigen were blocked in citrate buffer unmasking solution (Vector Laboratory) followed by incubation with blocking solution, and 18 h incubation at 4 °C with the following antibodies: cleaved caspase 3 (1:200) (Cell Signalling), PCNA (1:5000) (Cell Signaling), malon-dialdehyde (1:50) (Abcam). Visualization was performed using the ImmPact DAB peroxidase substrate kit (SK4105, Vector Laboratories) and counter-stained with haematoxylin (H-3401, Vector Laboratories). The slides were mounted using Vecta-Mount (H-5000, Vector Laboratories). Images were acquired using a Zeiss Axios microscope and analysed with ProRes Capture Pro 4.5 software.

Senescence-associated β-galactosidase staining. Senescence-associated β-galactosidase staining on mouse thymic lymphoma was performed as described previously24. Quantitative real time PCR. Total RNA was prepared from MEFs or mouse tissues using TRizol reagent (Invitrogen). Complementary DNA was synthesized from 5 μg of total RNA using the SuperScript III First-Strand Synthesis Kit (Invitrogen) according to the manufacturer’s protocol followed by qRT–PCR using the SYBR Fast qPCR master mix (Kapa Biosystems). qRT–PCR was performed using a ABI 7500 Fast Real-time PCR machine. Primers for mouse TaP63, ΔNp63, PUMA, Noxa, bax, PML, p16 and p21 (refs 34, 35) and human TaP63, ΔNp63 and GAPDH were used as described previously44. Human primers for PUMA, Noxa, bax, PML, P16, p16, p21 were used as described previously44 and GL2 and TIGAR as described previously44. Mouse primers for TaP63 are 5′-GCACCATCTTTCTGACCCTCCC-3′, R: 5′-GACAGTGAGCAAGATGGA-3′, Np63 are 5′-ATGTGGACTGCGTGGTACC-3′, R: 5′-GACCTGTGAGCAAGATGGA-3′, Jappare 5′-C TACCACTGTCTGATCGGTTGCC-3′, R: 5′-CCCTGGTTCACTCGCATGGGTGTT-3′. Human primers used for TaP63 are 5′-CAGCAGACCTACTTCTGACGTC3′, R: 5′-CCGCCACCCACACTATTA-3′ and for ΔNp63 are 5′-TTGACGGTGCAACTGACCTTT-3′. Similarly, putative ΔNp63 and ΔNp73 binding sites were scanned 1000 bp upstream of the 5′ UTR and in intron 2 of the IAPP gene. qRT–PCR was performed using primers specific for the indicated regions of IAPP. Promoter–Site 1 (−1800) forward: 5′-AGATGTCAGATATCGGCGACG-3′ and (−1731) reverse: 5′-TGTTTGGAGATGTCGCTCTCA-3′, Intron–2 Site 2 (−678) forward: 5′-GACAGGGATGCTTTAGGACC-3′ and (−765) reverse: 5′-CCTCAGGTGGATGCTCT-3′, and non-specific site (−7532) reverse: 5′-GTTGTTGAGTCTGTTGAGT-3′ and (−7623) reverse: 5′-ACACCTGATCTGCTGTGCTG-3′. Transfections and generation of IAPP- and hexokinase II-expressing cells. The mixture of siRNA and Lipofectamine were combined together and added to the well followed by the addition of 200,000 cells per well in a six-well dish. Transfections and generation of ΔNp73α and ΔNp63β transgenic mice. 3 × 105 cells were plated in 10-cm dishes. MEFs and human cancer cells were transfected with 8 μg Myc-DDK-IAPP (RC215074) (Origen) or 3.3 μg HKII (Plasmid 25529) (Addgene) using X-tremeGENE HP (Roche) and incubated for 48–72 h. Cells were selected with G418, MEFs (350 μg ml−1) and human cancer cells (500 μg ml−1) for a period of 9 days.
Secreted IAPP protein concentration. Twelve hours after knockdown of Np73/ΔNp73 in human cancer cells, fresh serum-free media was added to the cells. Following a sixty-hour incubation, the media was collected and concentrated using Amicon Ultra-15 Centrifugal Filter Units (UFC0901008, EMD Millipore).

RNA sequencing and analysis. Five micrograms of polyA+ RNA were used to construct RNA-Seq libraries using the standard Illumina protocol. Mouse mRNA sequencing yielded 30–40 million read pairs for each sample. The mouse mRNA-Seq reads were mapped using TopHat2 onto the mouse genome and build UCSC mm9 (UCSC Genome Browser) and the corresponding gene expression differences were computed using Cufflinks27. For each species, a combined profile of all samples was computed; mRNA abundance was mean-centred and Z-score transformed for each mRNA individually. Principal component analysis was executed using the implementation within the R statistical analysis system. Hierarchical clustering of samples was executed by first computing the symmetrical sample distance matrix using the Pearson correlation between mRNA profiles as a metric, supervised sample analysis was performed using the t-test statistics, and heat maps were generated using the heatmap.2 package in R. For gene signatures and pathway analysis gene list from the RNA-Seq comparing ΔNp73/ΔNp73- versus Np73δ4/ΔNp73- and Np73δ4/δp53−/− were obtained at a P value < 0.01. The genes upregulated in the Np73δ4/Δp53−/− and Np73δ4/δp53−/− were selected. The relative fold change of the genes were calculated and sorted from highest to lowest. Genes with a greater than 1.5-fold-increase were selected and run through the ingenuity pathway analysis (IPA) (Ingenuity Systems) to screen for pathways and processes. Genes from the selected pathways were cross-referenced with the Gene Set Enrichment (GSEA) (Broad Institute) data analysis, DAVID Bioinformatics Resource 6.7 and GSEA implementation at the Molecular Signature Database (MsigDB)28.

Magnetic resonance imaging. MRI imaging was performed at 10 weeks of age when the tumours were established and the weight range from 2.3 mm3 to 5 mm3. To reduce the variation between different groups of mice, a cohort of n = 5 with similar tumour volumes was established and tumours regression was monitored by MRI. All mice were scanned once a week for a period of 35 weeks on a 7-T, 30-cm bore MRI system (Bruker Biospin Co., Billerica, MA).

Hyperpolarized magnetic resonance spectroscopy. Dynamic MR spectroscopy (MRS) of hyperpolarized (HP) [1-13C]pyruvate was performed in vivo in tumour-bearing mice. To achieve polarization, a 26-mg sample of pyruvic acid (Sigma-Aldrich, St. Louis, MO) with 15 mM of OXO-0633 (red) (Ge Healthcare, Wausau, WI) and 1.5 mM Prohance (Braico Diagnostics Inc., Monroe Township, NJ), resulting in a final isotonic WI) and 1.5 mM Prohance (Bracco Diagnostics Inc., Monroe Township, NJ) was administered by intra-thymic injection (5 μl of running medium) to expose the sternum. Using a 28.5G U100 Insulin syringe, Adeno-mCherry/Adeno-Cre-mCherry (Gene Transfer Vector Core Facility, University of Iowa), Adeno-IAPP-mCherry (Vector Labs) or Adeno-shRNA-U6-mCherry (TRCN00000416196, Mission shRNA) (Vector Labs) (sequence CCCTGGTATTACCTCTGACGTCAG ATCCGAGTTCTAGCATGAGAATTTACATTTTGG) was surgically administered by intra-thymic injection (5 × 105 viral particles per gram of body weight) through the 2nd and 3rd sternum. The incision was sealed using wound clips and mice were allowed to recover. To determine the efficiency of the in vivo viral delivery to the thymic lymphoma, IVIS Lumina Imaging (Perkin Elmer) was performed 48 h later. Images were captured using a Mid-600 series bandwidth filter and analysed using the Living Image data analysis software.

In vivo human virus infection and IVIS Lumina imaging. All mice were anesthetized using isoflurane and 2% oxygen and placed on a custom bed. An incision was performed to expose the sternum. Using a 28.5G U100 Insulin syringe, Adeno-mCherry/Adeno-Cre-mCherry (Gene Transfer Vector Core Facility, University of Iowa), Adeno-IAPP-mCherry (Vector Labs) or Adeno-shRNA-U6-mCherry (TRCN00000416196, Mission shRNA) (Vector Labs) (sequence CCCTGGTATTACCTCTGACGTCAG ATCCGAGTTCTAGCATGAGAATTTACATTTTGG) was surgically administered by intra-thymic injection (5 × 105 viral particles per gram of body weight) through the 2nd and 3rd sternum. The incision was sealed using wound clips and mice were allowed to recover. To determine the efficiency of the in vivo viral delivery to the thymic lymphoma, IVIS Lumina Imaging (Perkin Elmer) was performed 48 h later. Images were captured using a Mid-600 series bandwidth filter and analysed using the Living Image data analysis software.

Apoptosis assay. Cells were plated at a density of 1 × 105 cells in 6 replicates in a 96-well dish. Twelve hours later, the cells were washed with 1× annexin-binding buffer and a cocktail of 5 μl annexin V–Alexa Fluor 488 for 100 μg ml−1 propidium iodide (PI) and 2 μg ml−1 Hoechst 33342 (Invitrogen) was added. Images were captured using a Zeiss Axio fluorescent microscope and analysed using the AxioVision Image 4.5 software.

Proliferation assay. The transfected human cancer cells were plated at a density of 1 × 105 cells in 6 replicates in a 96-well dish. Twelve hours later, the cells were labelled with 10 mM EdU (5−ethynyl-2′-deoxyuridine) for a period of 8 h. The assay was performed using the Click-IT EdU microplate assay (Invitrogen). Images were obtained using a Zeiss Axio fluorescent microscope and analysed using the AxioVision Image 4.5 software.

Glucone upturn measurement. Glucose uptake was calculated as a measure of glucose-dependent proton secretion from the maximum and basal glucose consumption after addition of 20 μl of 50 mM glucose and measured using the extracellular flux analyser (SeaHorse Biosciences XF96).

Glucose-6-phosphate assay. Glucose-6-phosphate was measured using a glucose-6-phosphate assay kit (ab88342, Abcam) following the manufacturer’s instructions. Forty-eight hours after transfection, 2 × 105 cells were collected, homogenized and passed through a 10-kDa spin-column filter. The eluate was collected and glucose-6-phosphate enzyme and substrate reaction was performed for 30 min and absorbance was measured at 450 nm.

In vitro adenovirus infection. ΔNp73δ4/Δp53−/− and ΔNp73δ4/δp53−/− MEFs were plated at a density of 2.5 × 105 cells in 10-cm dishes before infection. Twelve hours later, MEFs were infected with Adeno-CMV-mCherry or Adeno-CMV-Cre-mCherry (Gene Transfer Vector Core Facility, University of Iowa). The cells were infected at a multiplicity of infection of 6,000 particles per cell. The efficiency of infection was quantified by assessing mCherry-positive cells.

In vivo adenovirus infection and IVIS Lumina imaging. All mice were anesthetized using isoflurane and 2% oxygen and placed on a custom bed. An incision was performed to expose the sternum. Using a 28.5G U100 Insulin syringe, Adeno-mCherry/Adeno-Cre-mCherry (Gene Transfer Vector Core Facility, University of Iowa), Adeno-IAPP-mCherry (Vector Labs) or Adeno-shRNA-U6-mCherry (TRCN00000416196, Mission shRNA) (Vector Labs) (sequence CCCTGGTATTACCTCTGACGTCAG ATCCGAGTTCTAGCATGAGAATTTACATTTTGG) was surgically administered by intra-thymic injection (5 × 105 viral particles per gram of body weight) through the 2nd and 3rd sternum. The incision was sealed using wound clips and mice were allowed to recover. To determine the efficiency of the in vivo viral delivery to the thymic lymphoma, IVIS Lumina Imaging (Perkin Elmer) was performed 48 h later. Images were captured using a Mid-600 series bandwidth filter and analysed using the Living Image data analysis software.

In vivo knockdown. shRNA plasmids for Trp63 (Clone ID: Y31MM_5086894) (sequence TTGATCTCCGACACAGCTTCC) and Trpv3 (Clone ID: Y31MM_48857) (sequence TGCGAGTTGACAGATCCA) were obtained from the MD Anderson shRNA core facility (Open Biosystems). 293T cells were plated at a density of 2.5 × 105 cells in 10 cm dishes. Three micrograms of shRNA and packaging vectors were transcribed as described previously. Cells were selected using puromycin (3 mg ml−1) for 7 days.

In vitro and in vivo administration of 2-deoxy-D-glucose. 1 × 105 cells were plated in 6 replicate wells in a 96-well dish. Twelve hours later, the human cancer cells were treated with 50 mM final concentration of 2-deoxy-D-glucose (2DG) (D8375-5G, Sigma) for 1 h. Similarly, 2DG (500 mg per kg of tumour weight) (D8375-5G-Sigma) was administrated directly into the lymphoma of mice as described earlier29. N-acetyl-t-cysteine treatment. 1 × 105 cells were plated in 6 replicate wells in a 96-well dish. Twelve hours later, cells were treated with N-acetyl-t-cysteine (NAC) (2 mM) (A8199, Sigma) final concentration for a period of 1 h.

Aminyl and caspase inhibitor treatment. 2 × 105 cells were plated in triplicate in a 6-well dish. Twelve hours later, cells were treated with Aminyl peptide (5 μM) (A5972, Sigma) or with a caspase 1 inhibitor (20 μM) (Z-YVAD-FMK-218746, Calbiochem) for a period of 48 h.

In vivo and in vivo administration of pramintide acetate. 2 × 105 cells were plated in duplicate in a 6-well dish. Twelve hours later, cells were treated with 10 μg ml−1 pramintide acetate (AMYLIN Pharmaceuticals) or placebo for a period of 48 h. Pramintide acetate (AMYLIN Pharmaceuticals) or placebo (sodium acetate/acetate acid) was surgically administered through non-invasive intra-thymic injection using a multiple dose protocol of pramintide acetate (30 μg per gram of tumour weight). One injection per week for three weeks was administered directly into the thymic.
lymphoma of the animal. Another cohort of mice was treated bi-weekly for 3 weeks by intra-venous (IV) tail-vein injection of pramlintide acetate (45 µg per kg body weight) or placebo. The investigator was blinded to the treatment administered to each mouse. Tumour volumes were monitored weekly by MRI. Health and blood glucose levels of the treated animals were monitored weekly.

**In vitro and in vivo administration of calcitonin receptor antagonist.** $2 \times 10^5$ cells were plated in duplicate in a 6-well dish. Twelve hours later, cells were treated with Calcitonin receptor antagonist (1 nM) (AC187, Tocris Bioscience) for a period of 48 h with or without simultaneous pramlintide treatment. Similarly, a chronic dose of calcitonin receptor antagonist (1 nmol per gram of tumour weight) was administered through non-invasive intra-thymic injections with one injection every week for a period of three weeks with or without simultaneous pramlintide treatment. Tumour volume was monitored and measured weekly by MRI.

**Survival analysis.** Survival analysis was conducted for the IAPP, RAMP3 and CalCR gene in the following data sets: the Memorial Sloan Kettering Cancer Center and the TCGA Cancer cohort. We considered four major cancer types with high p53 mutation rates, which include lung squamous cell carcinoma, head and neck squamous cell cancer, basal breast cancer, and colon cancer. The co-expression of the three genes was analysed in cases only with p53 mutation. In all cases, we considered gene expression changes above or below two standard deviations with respect to the normal controls. The log-rank test and Cox $P$ test was used to assess significance between the samples with or without expression changes of the IAPP, RAMP3 and CalCR gene using the cBioPortal for cancer genomics.

**Statistics.** Sample size for mouse cohorts in each experiment was chosen based on the penetrance of the thymic lymphoma phenotype of the p53$^{-/-}$ mouse model (80%). Twenty to thirty mice were used for survival analyses. Data were analysed using a one-way ANOVA test or a Student’s $t$-test (two-sided) was used for comparison between two groups of data. A $P$ value of 0.05 was considered significant. Data are represented as mean ± s.e.m.

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Extended Data Figure 1 | Generation and characterization of ΔNp73 conditional knockout mice. a, The ΔNp73 targeting vector was generated by inserting loxP sites (triangles) flanking exon 3' and a neomycin cassette (neo) flanked by frt sites (squares). The location of PCR primers in each allele is shown by blue arrows. The targeted region of the floxed allele is depicted by yellow-dashed lines. b, Southern blot analysis using the 5' probe shown in a and tail genomic DNA derived from mice of the indicated genotypes. c, PCR analysis using tail genomic DNA of the indicated genotypes. d, Western blot analysis using mouse embryo fibroblasts (MEFs) of the indicated genotypes. e, f, qRT–PCR in MEFs of the indicated genotypes, n = 4, P < 0.005. Statistical significance is indicated by black asterisks.
Extended Data Figure 2 | Decreased thymic lymphomagenesis and increased survival in mice double deficient for ΔNp63 and p53 or ΔNp73 and p53. a, Quantification of thymic lymphoma incidence (n = 30 mice).

b, Table showing thymic lymphoma volumes. The difference in tumour volumes between p53−/− and ΔNp63−/−:p53−/− and p53−/− and ΔNp73−/−:p53−/− was statistically significant with P values, 0.03 and 0.002, respectively. c, Kaplan–Meier survival in mice. Boxed numbers indicate median survival. d, e, Western blot analysis of thymic lymphomas of the indicated genotypes. Arrows indicate specific isoforms, and asterisks indicate non-specific bands. f–h, qRT–PCR for PUMA (f), Noxa (g), and bax (h) in thymic lymphomas of the indicated genotypes, n = 4, P < 0.005.
i, Immunohistochemistry (IHC) for cleaved caspase 3 in thymic lymphomas. j, Quantification of apoptosis as assessed by cleaved caspase 3 staining, n = 20 fields of 3 biological replicates, P < 0.005. k–m, qRT–PCR for PML (k), p16 (l), and p21 (m) in indicated thymic lymphomas, n = 4, P < 0.005. n, IHC for PCNA in indicated thymic lymphomas. o, Quantification of the percentage of proliferation as assessed by PCNA staining, n = 20 fields of 3 biological replicates, P < 0.005. Statistical significance indicated by black asterisks.
Extended Data Figure 3 | Increased apoptosis and cell cycle arrest in ΔNp63<sup>+/−</sup>/p53<sup>+/−</sup> and ΔNp73<sup>+/−</sup>/p53<sup>+/−</sup> thymocytes after genotoxic stress. a. Western blot analysis in thymocytes derived from mice 6 h after treatment with 0 Gy or 10 Gy gamma irradiation. b–f, qRT–PCR for TAp63 (b), TAp73 (c), PUMA (d), Noxa (e), and bax (f) from samples shown in a, n = 4, P < 0.005. qRT–PCR normalized to samples treated with 0 Gy. 
g. Immunohistochemistry (IHC) for cleaved caspase 3 in samples from a. h. Quantification of the percentage of apoptosis as assessed by cleaved caspase 3 staining, n = 20 fields of 3 biological replicates, P < 0.005. i–k, qRT–PCR for PML (i), p16 (j), and p21 (k) using total RNA from samples shown in a, n = 4, P < 0.005. IHC for PCNA in samples shown in a. m, Quantification of the percentage of proliferation as assessed by PCNA staining, n = 20 fields of 3 biological replicates, P < 0.005. Statistical significance is indicated by black asterisks.
Extended Data Figure 4  | In vivo intra-thymic delivery of adenovirus-Cre-mCherry. a–c, IVIS Lumina imaging of thymic lymphomas of mice of the indicated genotypes infected with adenovirus (Ad)-mCherry (a) or Ad-Cre-mCherry (b, c) at 10 weeks of age and 48 h after adenoviral delivery. Red fluorescence indicates viral delivery to the thymus shown by the yellow dashed ovals. Red fluorescence near the mouth is due to auto-fluorescence of calcium and mineral deposits in the teeth. d, Western blot analysis using lysates from indicated thymic lymphomas 48 h after infection with adenovirus (Ad)-mCherry or Ad-Cre-mCherry. e, f, Quantitative real time (qRT–PCR) of thymic lymphomas 48 h after infection with Ad-mCherry (ΔNp63ΔA;p53−/− or ΔNp63ΔA;p53−/−) or Ad-Cre-mCherry (ΔNp63ΔA;p53−/− or ΔNp73ΔA;p53−/−), n = 4, P < 0.005. g, Immunohistochemistry (IHC) for cleaved caspase 3 in thymic lymphomas 48 h after infection with Ad-mCherry (ΔNp63ΔA;p53−/− or ΔNp73ΔA;p53−/−) or Ad-Cre-mCherry (ΔNp63ΔA;p53−/− or ΔNp73ΔA;p53−/−). h, Quantification of apoptosis as assessed by cleaved caspase 3 staining of the indicated thymic lymphomas, n = 20 fields of 3 biological replicates, P < 0.005. i, j, qRT–PCR of thymic lymphomas 48 h after treatment with Ad-mCherry (ΔNp63ΔA;p53−/−) or Ad-Cre-mCherry (ΔNp63ΔA;p53−/− or ΔNp73ΔA;p53−/−), n = 4, P < 0.005. k, Senescence-associated β-galactosidase (SA-β-gal) staining (blue) of thymic lymphomas 48 h after treatment with Ad-mCherry (ΔNp63ΔA;p53−/−) or Ad-Cre-mCherry (ΔNp63ΔA;p53−/− or ΔNp73ΔA;p53−/−). l–o, Flow cytometry plots of the indicated thymocytes at 4-week of age. p, Bar graph showing quantification of CD4, CD8, and CD4/CD8 double-positive (DP) cells. n = 3 mice per genotype, P < 0.005. q–s, Flow cytometry plots of thymic lymphoma cells 48 h after adenovirus-mCherry or adenovirus-CRE treatment for the indicated genotypes. t, Bar graph showing quantification of CD4, CD8, and CD4/CD8 double-positive (DP) cells in the indicated genotypes. n = 3 mice per genotype, P < 0.005. u, Cartoon representation of isolation of CD45-positive thymic lymphoma cells from 10-week-old mice of indicated genotypes. v, Western blot analysis of CD45-positive thymic lymphoma cells after treatment with Ad-mCherry (ΔNp63ΔA;p53−/−) or Ad-CRE-mCherry (ΔNp63ΔA;p53−/− and ΔNp73ΔA;p53−/−). Statistical significance is indicated by black asterisks.
Extended Data Figure 5 | Loss of ΔNp63/ΔNp73 induces TAp63 and TAp73 upregulation in the absence of p53.  a, Western blot analysis in ΔNp63*/p53−/− MEFs before (ΔNp63+/p53−) and after (ΔNp63+/ΔNp63−/p53−) Ad-Cre administration. b, c, qRT–PCR for ΔNp63 in indicated MEFs. d, Western blot analysis in ΔNp73+/ΔNp73−/p53−/− MEFs. e, f, qRT–PCR for ΔNp73 in indicated MEFs. g, Table showing ΔNp63 and ΔNp73 binding sites on the TAp63 and TAp73 promoter regions. h, i, qRT–PCR of chromatin immunoprecipitation using indicated MEFs and an antibody for p63 (h) or p73 (i) n = 3, P < 0.005. j, k, Western blot analysis in ΔNp63+/p53−/− or ΔNp73+/ΔNp73−/p53−/− MEFs treated with the indicated shRNAs (shNT) indicates a non-targeting scramble shRNA. l–q, qRT–PCR for PUMA (l), Noxa (m), bax (n), PML (o), p21 (p), and p16 (q) in the indicated MEFs expressing the indicated shRNAs, n = 5, P < 0.005. Statistical significance indicated by black asterisks.

**Table: ΔNp63/ΔNp73 binding sites on TAp63 and TAp73 promoter**

| Element     | Location | Sequence             | MM/Spacer |
|-------------|----------|----------------------|-----------|
| TAp63-Promoter | -43 to -18 | tgaCAGGagc tctca aatCAAGtca | 5/5       |
| TAp73-Promoter | -1106 to -1082 | cgcCTAGcac acca atlCAAGgaa | 8/4       |

Mismatches are shown in red text. MM: the number of mismatches in each binding site. Spacer: number of nucleotides between two half sites.
Extended Data Figure 6 | Metabolic genes including IAPP are upregulated in thymic lymphomas deficient for ΔNp63 or ΔNp73 and p53. a, Supervised hierarchical clustering of RNA-sequencing data from thymic lymphomas 48 h after treatment with Ad-mCherry (ΔNp63 Δp53−/−) or Ad-Cre-mCherry (ΔNp63Δ/Δp53−/− or ΔNp73Δ/Δp53−/−). b, c, qRT–PCR for GLS2 (b) and TIGAR (c) in the indicated thymic lymphomas, n = 4, P < 0.005. d, qRT–PCR for GLS2 in MEFs of the indicated genotypes expressing shRNAs for a non-targeting sequence (shNT), TAp63 (shTAp63) and TAp73 (shTAp73), n = 4, P < 0.005. e, Table showing the TAp63 and TAp73 binding sites on the IAPP promoter and intron 2. f, g, qRT–PCR of promoter site 1 using chromatin immunoprecipitation in MEFs of the indicated genotypes, n = 3, P < 0.005. h–k, Dual luciferase reporter assay for pGL3-IAPP-promoter site 1 (h, i) and a mutant version of this reporter gene (pGL3-IAPP MUT) (j, k). Genotypes of MEFs and vectors used are shown. V represents pcDNA3 vector. l, m, Western blot analysis of the indicated MEFs expressing IAPP or siRNAs for a non-targeting sequence (siNT) or IAPP (siIAPP). Statistical significance indicated by black asterisks.
Extended Data Figure 7 | Systemic in vivo delivery of pramlintide results in tumour regression in p53-deficient thymic lymphomas. a, Western blot analysis showing IAPP expression in the indicated thymic lymphomas, n = 5 mice. b, Kaplan–Meier survival indicating thymic lymphoma-free survival. n = 8 mice per group, P < 0.005. c, Cartoon indicating schedule of MRI imaging and injection (Inj.) of pramlintide in mice with p53-deficient thymic lymphomas. d–q, MRI imaging at 10, 11, 12 and 13 weeks after treatment with placebo (d–g) or pramlintide (i–p); quantification of tumour volumes in placebo (n = 3) (h) and pramlintide-treated mice (n = 7) (q), P < 0.005. Statistical significance indicated by black asterisk.
Extended Data Figure 8 | IAPP inhibits glycolysis by increasing intracellular G-6-P levels. a, b, Quantification of apoptosis (a) and proliferation (b), n = 20 fields of 3 biological replicates, P < 0.005. c, qRT–PCR for the target genes indicated on the x-axis in the indicated H1299 cells expressing the indicated siRNAs, n = 4. Asterisks indicate statistical significance (P < 0.005) relative to siNT. d, Western blot analysis of H1299 cells treated with the indicated siRNAs. e, f, Bar graph indicating glucose-dependent proton secretion as a measure of glucose uptake and intracellular levels of glucose-6-phosphate in H1299 cells with the indicated siRNAs and treatments (f). g, Colour-coded legend for panels e, f and i. h, Western blot analysis of H1299 cells expressing the indicated siRNAs. i, Immunofluorescence analysis for ROS (red) or apoptosis (green or green/red) in H1299 cells expressing the indicated siRNAs and treated with 2DG and/or NAC.
Extended Data Figure 9 | Treatment of p53-mutant human cancer cell lines with pramlintide inhibits glycolysis and induces ROS and apoptosis.
a, b, Western blot analysis of H1299 cells expressing the indicated siRNAs (a) or concentrated media derived from H1299 cells expressing siNT, siΔNp63, or siΔNp73 (b). c, Extracellular acidification rate (ECAR) using H1299 cells expressing the indicated siRNAs and treated with the indicated media containing secreted IAPP and treated with the indicated amylin inhibitor (AI).

d–g, Extracellular acidification rate (ECAR) as a measure of glycolysis in SW480 (d), MDA-MB-468 (e), SRB12 (f) and COLO16 (g) human cancer cell lines after treatment with placebo, pramlintide, or pramlintide and a calcitonin receptor inhibitor (CalR I.), n = 3, P < 0.005. Glucose, oligomycin, and 2-deoxy-D-glucose (2DG) were supplied to the media at the indicated time points shown on the x-axis.

h, i, Immunofluorescence for ROS (red) (h) and apoptosis (green) (i) on the indicated cells, n = 3.

j, k, Kaplan–Meier survival curves using data from patients with p53 mutant tumours with the indicated cancers and co-expression of IAPP, RAMP3 and CALCR. Boxed numbers represent median survival.