An *in vivo* KRAS allelic series reveals distinct phenotypes of common oncogenic variants

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ABSTRACT

KRAS is the most frequently mutated oncogene in cancer. Tumor sequencing has revealed a complex spectrum of KRAS mutations across different cancer types, yet there is little understanding how specific KRAS alterations impact tumor in initiation, progression, or therapy response. Using highfidelity CRISPR-based engineering, we created an allelic series of new LSL-Kras mutant mice, reflecting codon 12 and 13 mutations that are highly prevalent in lung (KRAS^{G12C}), pancreas (KRAS^{G12R}) and colon (KRAS^{G13D}) cancers. Induction of each mutation in the developing mouse pancreas reveal striking quantitative and qualitative differences in the degree of ductal transformation and pre-malignant progression. Further, using organoid models we show that KRAS^{G13D} mutants respond to EGFR inhibition, while the anti-proliferative effect of KRASG12C-selective inhibitors can be overcome by upstream EGFR signaling. Together, these new mouse strains provide an ideal for investigating KRAS biology in vivo, and for developing pre-clinical precision oncology models of KRAS-mutant pancreas (G12R), colon (G13D), and lung (G12C) cancers.

KRAS is the most frequently mutated oncogene in human cancers and considered a key early driver of many tumors. Specific cancer types show a clear bias in the types and frequency of KRAS alterations^{1,2}, and while carcinogenspecific mutational signatures define a subset of tissueselective KRAS changes, they do not account for the majority of tissue-selective KRAS alterations^{3,4}. Biochemically, oncogenic KRAS mutations increase the abundance of GTPbound 'active' KRAS protein, but different amino acid changes can significantly alter the kinetics of GDP/GTP exchange and GTP hydrolysis⁵. Such changes may have implications for signaling dynamics in different cell or tissue contexts. Finally, mounting clinical and pre-clinical evidence suggests that tumors carrying distinct KRAS variants are differentially sensitive to targeted therapies⁶⁻⁸. Thus, despite genetic and epidemiologic evidence that differences between KRAS mutations are functionally important, we still do not have a clear understanding of how distinct KRAS alterations dictate tumor initiation, disease progression, or response to therapy.

Conditional animal models, such as *Lox-Stop-Lox* (*LSL*)-*Kras*^{G12D} and *LSL-Kras*^{G12Vgeo} mice developed almost 20 years ago^{9,10}, have been critical tools to dissect the role of

KRAS mutations in tumor development. However, these models alone do not recapitulate the spectrum of KRAS alterations in human cancer. Here we describe an efficient pipeline for engineering allelic series of conditional alleles that significantly expands repertoire of pre-clinical KRASdriven cancer models. Using high-fidelity CRISPR targeting in embryonic stem cell (ESC)-based mouse models (GEMM-ESCs)¹¹⁻¹⁴, we engineered six new LSL-Kras mutant alleles (G12V, G12C, G13D, G12R, G12A, G12S) that represent the most frequent mutations at the G12/G13 hotspot, after G12D. Guided by clinical data, we generated conditional mice representing three tissue-selective alterations observed in colorectal (G13D), pancreatic (G12R), and lung cancer (G12C) and show that, even subtle mutational changes in the Kras oncogene, have a profound impact on tumor initiation in the pancreas.

In line with the diverse biological outcomes *in vivo*, pancreatic organoids derived from these models uncovered mutant Kras variant-specific vulnerabilities that render KRAS^{G13D} mutant cells sensitive to epidermal growth factor receptor (EGFR) inhibition and that support synergy between RTK inhibition and active KRAS^{G12C} inhibitors. Thus, these new animal models serve as a powerful pre-clinical resource to interrogate KRAS biology in vivo and develop rational strategies to effectively target specific KRAS mutant cancers.

RESULTS

A CRISPR-based pipeline for engineering Kras allelic variants

To engineer new *LSL-Kras* mutants, we used CRISPR-mediated homology directed repair (HDR) to introduce specific codon 12/13 mutations into the well-characterized *LSL-Kras*^{G12D} allele. For this, we took advantage of previously derived genetically engineered embryonic stem cells (GEMM-ESCs)¹⁴ carrying the endogenous *LSL-Kras*^{G12D} allele, with a pancreas specific Cre recombinase (*Ptf1a-Cre*, also known as *p48-Cre*) and a far-red fluorescent Cre-reporter (*CAGs-LSL-rtTA3-IRESmKate2*, hereafter *LSL-mKate2*). The GEMM-ESC approach enables the rapid creation of mouse cancer models without the need for extensive intercrossing to generate appropriate genotypes, which is particularly important when modeling multiple new genetic modifications that may take many years to breed^{11,13,14} (Figure 1a). Alleles derived from GEMM-ESC can be outcrossed from the founder generation to establish

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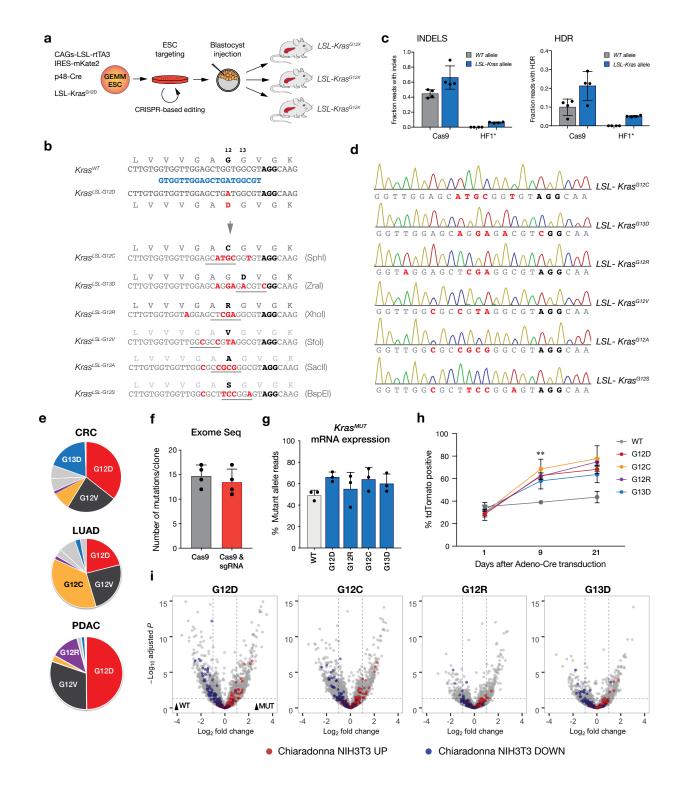


Figure 1. CRISPR strategy to generate new conditional Kras variants. a. Schematic displaying the pipeline followed to generate the new LSL-Kras alleles were created by modifying the existing LSL-KrasG12D allele, via CRISPR-based gene editing in embryonic stem cells (ESCs) carrying a LSL-Kras variant, a pancreas specific Cre (p48-Cre) and far-red fluorescent reporter b. ESCs were co-transfected with a vector expressing both Cas9 and Kras-targeted sgRNA, together with one-single stranded donor oligonucleotide (ssODN) template bearing the new mutation. c. Targeted deep sequencing from ESCs bulk population after co-transfection. Error bars= SD, n=4 independent transfections. d. Sanger sequencing traces from ESCs clones carrying the new Kras mutation. e. Pie charts representing the mutational Kras codon 12 and 13 spectrum in colorectal cancer (CRC), lung adenocarcinoma (LUAD) and pancreatic adenocarcinoma (PDAC). f. Whole exome sequencing data from ESC clones transfected with Cas9-Hfc alone or with ssODN. Error bars (n=4 independent clones in each Kras mutant or wild-type (WT) murine embryonic fibroblast (MEF) lines obtained by transcripts per million estimates. Error bars, (n=3 independently generated MEF lines from each genotype) h. Competition assay in MEFs showing relative abundance of tdTomato-positive cells after Adeno-Cre delivery. Error bars= SD, n=3 independently generated MEF lines from each genotype, **p value < 0.01 between G12D/G12R vs WT at day 9 post infection. i. Volcano plots from MEF RNAseq data comparing all mutants with a published gene set (Chiaradonna et al, 2016) (n=3 independently generated MEF lines from each genotype)

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independent strains.

We first designed an sgRNA overlapping codons 12 and 13 of Kras and 140mer single-stranded DNA (ssDNA) HDR templates carrying each specific mutation (Figure 1b), and introduced them into ESCs by nucleofection. For screening and genotyping purposes, each ssDNA template was designed to carry silent mutations that generate a unique restriction site adjacent to the specific codon change (Figure 1b). Analysis of the bulk transfected population revealed efficient generation of indels in both the LSL-G12D and wildtype Kras alleles, suggesting that the single mismatch between the sgRNA and target region in WT Kras was not sufficient to dictate allele selectivity (Figure 1, Supplementary Figure 1a). Indeed, every clone we identified that carried the desired HDR event on the LSL allele, carried an indel in the WT coding sequence (14/14 clones sequenced). Our follow-up efforts to first introduce silent mutations in the WT allele and then retarget the LSL-Kras^{G12D} site also failed due to the introduction of indels in Nras with the Kras WT-specific sgRNA (Supplementary Figure 1b-d).

During the course of this work, we independently developed an expression-optimized high-fidelity Cas9 variant that enabled selective and potent genome targeting ^{15,16}. We thus tested whether this would provide the specificity required for selective LSL-Kras targeting. In contrast to what we observed following transfection of wildtype Cas9, expression of the optimized HF1 enzyme resulted in the generation of both indels and HDR integration in the LSL allele, but induced no detectable modifications in WT Kras (Figure 1c, Supplementary Figure 2a). Though we noted an overall decrease in the efficiency of HDR-targeting in comparison with wildtype Cas9 (5% vs 21%), the increased specificity allowed the identification of numerous clones carrying the desired targeting event (Supplementary Table 1). Targeted deep sequencing of Kras exon 2 revealed a consistent 5-6% HDR frequency, though the number of individual positive clones identified following each transfection varied from 1-5%, due to random clone selection (Table 1). Clones identified to carry integration of the donor template by restriction digest were confirmed by allele specific PCR and direct Sanger sequencing (Figure 1d, Supplementary Figure 2b).

To determine the impact of selective KRAS mutational variants on cell and tumor biology, we chose to generate mice from three *LSL-Kras* genotypes: *LSL-Kras*^{G12C}, *LSL-Kras*^{G12R}, and *LSL-Kras*^{G13D}, as these mutations represent frequent and tissue restricted mutational events in human lung (LUAD), pancreatic (PDAC), and colorectal cancer (CRC), respectively (Figure 1e). To confirm that CRISPR-mediated HDR-targeting had not caused widespread mutagenesis or large-scale chromosome aberrations, we performed whole-exome sequencing (WES) on selected ESC clones, and those transfected with only Cas9 or the expression-optimized HF1 Cas9 variant (no sgRNA). Consistent with highly selective targeting of the *Kras* locus by this sgRNA, we observed no difference in the number of de novo mutations (~14 mutations/clone) in cells transfected with either Cas9 alone, or Cas9/sgRNA (Figure 1f, Supplementary Table 1). Moreover, most mutations were single nucleotide variants, rather than indels usually seen with Cas9-mediated mutagenesis, suggesting they arose spontaneously during ESC culture. Further, we did not detect any chromosome copy number alterations, with the exception of one *LSL-Kras^{G13D}* clone, that showed a small deletion on chromosome 4 (Supplementary Figure 3a); this clone was not used for mouse generation.

To produce mice, targeted LSL-Kras^{mut} ESC clones were injected into host albino C57Bl/6J blastocysts, creating a range of high contribution chimeras (Supplementary Figure 3b); we further bred the founders to C57Bl/6N mice to increase the number of animals for this study. To first confirm that each of the new strains showed equivalent expression of the Kras mutant allele, we generated multiple independent murine embryonic fibroblasts (MEFs) cultures from each LSL-Kras^{MUT} line and immortalized the cells by disruption of p53 with CRISPR. Delivery of Cre recombinase on the same vector as Cas9 (Cas9-P2A-Cre) enabled simultaneous induction of each Kras^{MUT} allele. As expected, all mutant alleles were expressed similarly, ranging from 59 to 66% of total Kras transcript (Figure 1g). Consistent with previous analysis of KRAS^{G12D} MEFs¹⁷, induction of endogenous KRAS mutations in p53 wildtype cells led a proliferative advantage, but there was no significant difference in proliferation between MEFs carrying each different KRAS mutant (Figure 1h). RNAseq analysis in Kras^{mut}/Trp53^{KO} MEFs, revealed a range of transcriptional changes between WT and KRAS^{MUT} cells, including the up and down regulation of genes previously linked to KRASdriven transformation in murine fibroblasts (Figure 1i)¹⁸. Notably, though each of the KRAS variants carried the mutant transcriptional signature, the magnitude of the effect was markedly reduced in KRAS^{G12R} and KRAS^{G13D} cells (Figure 1i). Together, these data show that each LSL-Kras^{MUT} strain enables comparable induction of endogenous *Kras^{MUT}* alleles, and that while each mutation drives KRAS-associated phenotypes, the downstream consequences of individual codon 12/13 KRAS mutations are not identical.

Distinct Kras alterations has diverse consequences for tumor initiation in the pancreas

KRAS mutations are a near universal feature of pancreatic ductal adenocarcinoma (PDAC) and are a considered the key initiating event in this disease14,19-21. Induction of KRASG12D or KRAS^{G12V} mutations in the developing epithelium of the mouse pancreas drives widespread transdifferentiation of the acinar compartment (acinar to ductal metaplasia; ADM) and the development of premalignant pancreatic intraepithelial neoplasias (PanINs)²⁰⁻²². To determine how distinct oncogenic alterations in codons 12/13 of KRAS impact tumor initiation in the pancreas, we analyzed LSL-Kras^{MUT}/Ptf1a-Cre (KC) mice carrying each different Kras^{MUT} allele. In this model, Cre is expressed mid-gestation and activates KRAS^{MUT} expression in almost all epithelial cells of the pancreas^{14,23} (Supplementary Figure 4a). By 4 weeks of age, changes were apparent in the pancreas of all genotypes, with evidence of ADM and early PanIN development (Supplementary Figure

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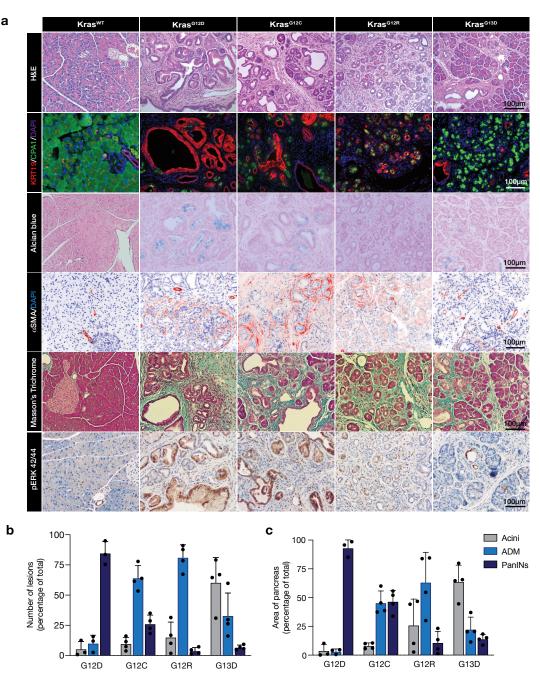


Figure 2. Tumor initiation in pancreas displays a different phenotype in each Kras mutant strain. a. Histological cross-sections, immunofluorescent and immunohistochemical stains of 12-week-old pancreata from each Kras mutant strain, as indicated. Graphs show number (b) and area (c) of pancreatic lesions quantified as acini, acinar-to-ductal metaplasia (ADM) or pancreatic intraepithelial neoplasias (PanlNs) (n=3-4 mice per genotype).

4b). Transcriptome analysis of whole pancreatic tissue at this time revealed gene signatures consistent with KRAS activation in the pancreas, including upregulation of genes involved in epithelial to mesenchymal transition (EMT), KRAS signaling, and inflammatory responses (Supplementary Figure 5a). Overall, altered gene sets were similar between all mutants at early this time-point (Supplementary Figure 5b), though the increase in ductal (epithelial) and fibroblast markers, and corresponding decrease in the expression of genes marking acinar cells was reduced in G12R and G13D variants, relative to G12D and G12C (Supplementary Figure 5c).

By 12 weeks of age, *LSL-G12D* mice showed the expected appearance of PanIN lesions with loss of the acinar marker Carboxypeptidase A1 (CPA1), SOX9 induction²⁴, expression of the ductal lineage cytokeratin, KRT19, and the production

of mucins (Alcian Blue) (Figure 2a,b). Accompanying this change was the infiltration of alpha smooth muscle actin (α SMA) positive stromal cells (α SMA; Figure 2a) and ectopic deposition of extracellular matrix (Figure 2b; Masson's Trichrome, blue staining). As expected, PanIN lesions showed an increase in phosphorylation of the downstream MAPK effectors, ERK1/2 (pERK1/2; Figure 2b). Interestingly, *LSL-G12C* mice showed similarly elevated pERK, yet the overall PanIN burden was reduced, with the remainder of the pancreatic epithelium either normal acinar tissue or undergoing ADM (Figure 2a,b). In contrast, *LSL-G13D* pancreata appeared predominantly histologically normal, with less than one third of the pancreas showing evidence of ADM or PanIN transition. However, those regions containing PanINs closely resembled G12D or G12C lesions, containing increase

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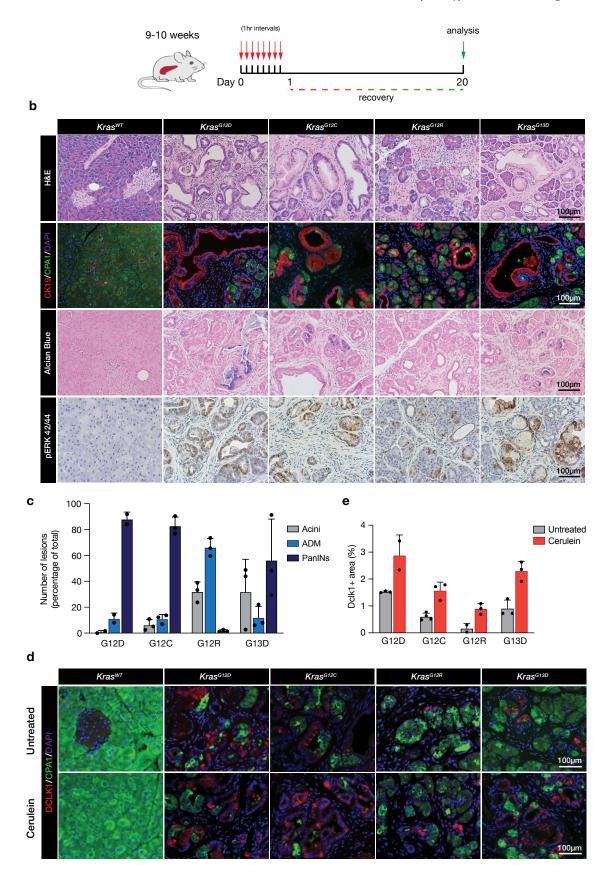


Figure 3. Cerulein-induced pancreatitis accelerates tumor progression in G12C and G13D KRAS mutants, but not G12R a. Schematic depiction of cerulein acute pancreatitis treatment. b. Histological cross-sections, immunofluorescent and immunohistochemical stains of 12-week-old pancreata (20 days following cerulein treatment) from each Kras mutant strain, as indicated. c. Graphs show number (b) and area (c) of pancreatic lesions quantified as acini, acinar-to-ductal metaplasia (ADM) or pancreatic intraepithelial neoplasias (PanINs) (n=2-3 mice per genotype). d. Immunofluorescent staining for DCLK1 in pancreatic tissue sections from untreated or cerulein-treated mice. e. Percentage of DCLK1+ stained area (n=2-3 mice per genotype).

matrix deposition and downstream MAPK activation (Figure 2a; Masson's Trichrome, blue staining; pERK staining, brown color). Similar to G12D and G12C epithelium, G12R lesions displayed early responses to KRAS activation, including elevated SOX9 expression²⁴ (Supplementary Figure 6), but in contrast, showed almost no progression to PanIN (Figure 2b); G12R pancreata had no evidence of Alcian Blue staining, and the epithelium showed expression of both acinar (CPA1) and ductal (KRT19) markers, suggesting a stalled progression at ADM (Figure 2a, c-d). Lack of phenotypic progression in G12R and G13D mice was also evident at 24 weeks (Supplementary Figure 4b), suggesting that the differences observed were not simply due to a moderate slowing of disease course. Together, these results show that KRASG12C, KRASG12R and KRASG13D mutations confer a different disease initiating capacity in comparison with the well-studied Kras^{G12D} mouse model in pancreas, and suggest that these contrasting features may underlie the enriched penetrance of KRASG12D mutant variants observed in PDAC.

Acute pancreatitis promotes progression of G12C and G13D, but not G12R preneoplastic lesions.

In patients, chronic pancreatitis substantially increases the risk of developing PDAC²⁵, and similarly, induction of acute pancreatitis in mice by high doses of the cholecystokinin (CCK) analogue, cerulein, promotes the early progression of disease ^{22,26,27}. To determine whether acute pancreatitis would alter the progression of pancreatic precursors carrying different KRAS mutations, we treated 9-week old mice with cerulein (8 doses, 50µg/kg, 1 hour apart), and assessed pancreatic response 20 days following injury (Figure 3a). As expected, WT mice showed full recovery of the acinar tissue, while LSL-G12D mice contained almost no normal acinar tissue, with the majority of the pancreas made up of PanINs (Figure 3b). Both LSL-G12C and LSL-G13D mice showed a marked progression of disease, with LSL-G12C pancreata containing more than 90% PanINs, while LSL-G13D animals switched from >65% normal acinar tissue, to >65% PanIN lesions (Figure 3c). Each of the LSL-G12D, LSL-G12C and LSL-G13D mice showed a more severe phenotype than untreated mice, with evidence of inflamed areas, atrophy, and the presence of cysts (Figure 3b). Strikingly, cerulein treated LSL-G12R pancreata appeared similar to untreated mice, with the majority of tissue stalled at ADM stage, and less than 5% of the pancreas containing PanIN lesions (Figure 3b-c).

Progression of KRAS^{G12D}-mutant preneoplastic lesions in the pancreas both before and following cerulein-induced pancreatitis requires the induction of a quiescent stem cell population marked by expression of Doublecortin-like kinase 1 (DCLK1) ^{28,29}. To test whether G12R mutant animals had a selective defect in the induction of this stem population, we quantified the frequency of DCLK1-positive cells in untreated and cerulein-treated pancreata. As expected, like G12D, both G12C and G13D pancreata showed relatively abundant DCLK1-positive cells that were increased following cerulein treatment (Figure 3d). In contrast, G12R pancreata contained almost no DCLK1-positive cells, and while the number increased following pancreatitis, it remained lower than all other genotypes (Figure 3e). Together, these data show that distinct KRAS mutations have both a quantitative and qualitative impact on the pre-malignant transformation of the pancreatic epithelium. The specific failure of KRAS^{G12R} mutant pancreatic epithelium to transition from metaplastic acini to PanIN lesions is unexpected, given the frequency of KRAS^{G12R} mutation in human pancreatic cancer, but maybe linked to a reduction in the DCLK1-positive regenerative stem cells that are important for disease progression in the pancreas²⁸.

Distinct KRAS mutations induce differential sensitivity to EGFR inhibition

To assess tumor-cell intrinsic differences in between KRAS mutations and explore the potential for these new strains as effective tools for testing therapeutic interventions, we derived ductal pancreatic organoids from each LSL-Kras^{MUT} mice and induced simultaneous Cre-mediated Kras activation and p53 disruption by CRISPR (KP organoids). Targeted deep sequencing of the Trp53 locus following Nutlin3 selection confirmed frameshift alterations in greater than 99.9% of all lines (Supplementary Figure 7). Pancreatic organoids from KP tumors have been previously well-characterized and reflect an accurate ex vivo surrogate for murine PDAC²⁹. We analyzed the transcriptional profile of each KP mutant line and compared it to previously reported KP organoids²⁹. Similar to MEFs, G12C (KP) organoids closely mimicked the transcriptional changes observed both in our G12D cells and previously published tumor-derived G12D organoids²⁹. Again, similar to MEFs and pancreatic tissue, both G12R and G13D organoids showed fewer differentially expressed genes compared to WT organoids, and Kras^{G12D}-linked gene expression changes were substantially reduced (Figure 4a).

Current clinical guidelines exclude all patients with KRAS mutations from treatment with small molecules or antibodies that target the epidermal growth factor receptor (EGFR). However, retrospective clinical data has suggested that colorectal cancers carrying KRAS^{G13D} mutations may be sensitive to Cetuximab^{6,7}. G12D KP organoids showed decrease phosphorylation of downstream effectors ERK1/2 and Akt (Figure 4b) following Gefitinib treatment, suggesting endogenous upstream RTK signaling was important for maintaining high levels of MAPK signaling in organoid. Despite this, Gefinitib induced minimal antiproliferative response in G12D organoids, and they could be maintained indefinitely in the presence of drug (Figure 4 c-e). In contrast, G13D KP organoids showed a profound cell cycle arrest within 48 hours of Gefitinib treatment (Figure 4c-d), and could not survive long-term (>1 week) drug exposure (Figure 4e). Both G12C and G12R KP organoids had an intermediate response to Gefinitib, but ultimately were able to maintain growth and proliferation with extended treatment (Supplementary Figure 8).

Activating KRAS mutations are rarely observed with other MAPK activating alterations, such as mutational activation of BRAF, NRAS or EGFR³⁰; or loss of negative regulators like the GTPase activating protein Neurofibromin 1 (NF1) ³¹ (Figure

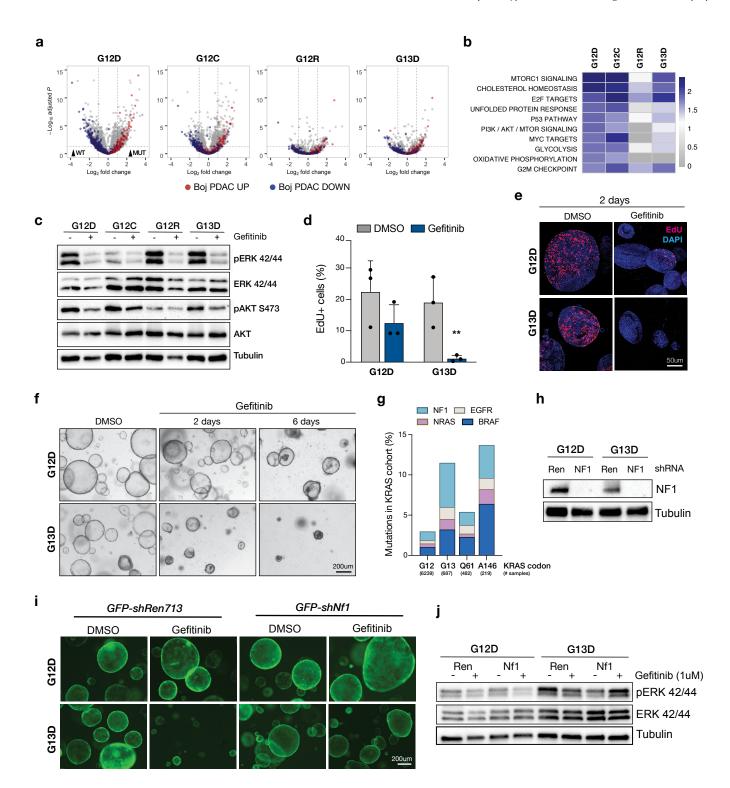


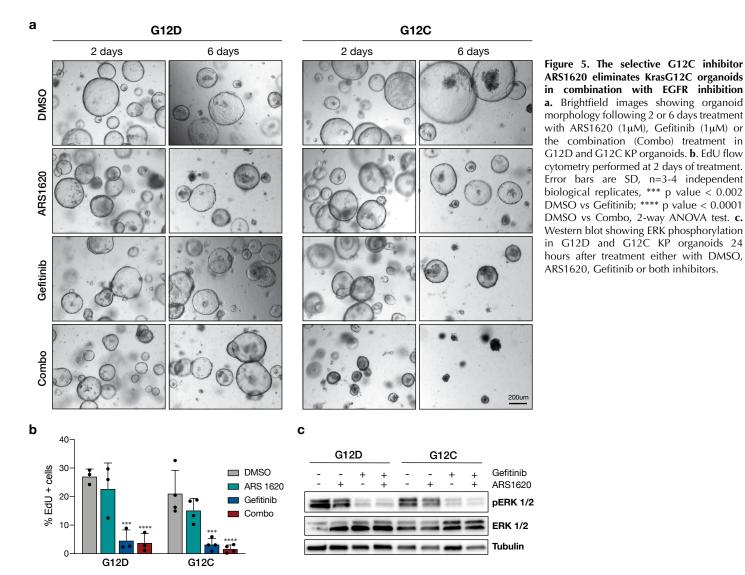
Figure 4. KrasG13D pancreatic organoids are sensitive to EGFR inhibiton via an NF1-dependent process. a. Volcano plots of Kras^{MUT}/p53^{MUT} (KP) organoids compared to Kras^{WT}/p53^{MUT} organoids, showing expression of previously published PDAC gene signature ²⁹. **b.** Gene set enrichment analysis summary displaying the top 10 enriched pathways in Kras^{G12D} organoids, compared to KrasWT organoids. **c.** Western blots performed in G12D, G12C, G12R and G13D KP organoids following 2 days of Gefitinib or DMSO treatment, showing a decrease in phospho-ERK 42/44 signaling after EGFR inhibition. Immunofluorescent images (**d**) and EdU flow cytometry (**e**) from Gefitinib or DMSO-treated G12D and G13D KP organoids, treated with Gefitinib or DMSO (2 days). Proliferating organoids are marked by EdU (red) in the images. Error bars are SD, n=3 independent biological replicates, ****** p-value < 0.005 DMSO vs Gefitinib; 2-way ANOVA. **f.** Brightfield images from G12D and G13D KP organoids treated with DMSO or 1µM Gefitinib for 2 and 6 days. **g.** Co-mutation data for NF1, EGFR, NRAS, and BRAF in KRAS mutant cancers, sub-divided by codon position, G12, G13, Q61 and A146. Data obtained from the Genomic Evidence Neoplasia Information Exchange (GENIE) dataset. **h.** Western blot showing NF1 knockdown or control shRenilla.713 ("Ren") G12D and G13D KP organoids. **i.** shNF1 and shRen expressing G12D and G13D organoids treated with DMSO or Gefitinib for 3 days. GFP is linked to expression of the shRNA. j. Western blot displaying an increase in ERK 42/44 phosphorylation in Gefitinib-treated G13D organoids after NF1 knockdown.

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4f, Supplementary Figure 9a). Recent work highlighted a subset of BRAF mutations (Class III) that require upstream RAS signaling to induce high levels of MAPK activation, and often co-occur with other RAS-MAPK mutations. Similarly, analysis of ~55,000 cancers in the Project Genie database ³² showed that tumors carrying KRAS codon 13 mutations are four times more likely to have additional driver mutations in MAPK genes (Figure 4f, Supplementary Figure 9b). In particular, G13D and G13C mutant cancers showed 5-fold enrichment in truncating NF1 mutations (Figure 4f), suggesting that these mutant proteins may be subject to upstream RTK/RAS and/or NF1 regulation. To test this hypothesis directly we silenced NF1 in KP pancreatic organoids (Figure 4g-h) and treated them with Gefitinib for 3 days (Figure 4h). G13D KP organoids transduced with a control hairpin (shRen 713) remained sensitive to EGFR inhibition while NF1-silenced organoids showed elevated pERK and continued expansion in the presence of drug (Figure 4i). As expected G12D organoids showed no change in growth following EGFR inhibition, regardless NF1 expression (Figure 4h). These data are consistent with recent reports of KRASG13D mutant cancer cell line vulnerabilities^{33,34}.

EGFR inhibition reveals the effect of KRAS G12C covalent inhibitors

The recent development of covalent inhibitors of KRASG12C represents the first strategy to directly target oncogenic KRAS, and multiple small molecules have shown promise in early stage clinical trials^{35,36}. To determine whether our *LSL-Kras^{G12C}* model is an effective pre-clinical tool to investigate response and resistance to clinical G12C inhibitors, we treated organoids with ARS1620 - a selective KRASG12C inhibitor that covalently binds to Cys12 when KRAS is in its GDP-inactive state ³⁷. Surprisingly, like G12D organoids, G12C cells were insensitive to the G12C inhibitor, showing no change in EdU incorporation at 2 days. However, KRAS^{G12C} KP organoids were uniquely sensitive to a combination of ARS1620 and Gefitinib (Figure 5a-c). These data indicate that upstream signaling by RTKs can impact the outcome of downstream G12C inhibition, in line with recent reports in cell lines and PDX models^{36,38,39}. These findings highlight the fidelity of which the LSL-Kras^{G12C} allele recapitulates key signaling and feedback regulation observed in human cancer cells.



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DISCUSSION

Mutant KRAS is a clear driver of human cancer and consequently there are numerous FDA-approved and investigational drugs in clinical use, targeting KRAS and downstream effectors. Owing to expansive tumor resequencing efforts, we know the types and frequency of KRAS alterations in different cancer types in great detail. In contrast, how each distinct mutation impacts disease initiation and progression remains unknown. Understanding the similarities and differences in cancerassociated KRAS alleles may reveal unique dependencies that can be exploited therapeutically. Here, we report a highfidelity CRISPR engineering strategy to generate an allelic series of mice bearing unique conditional KRAS mutations. Using these new pre-clinical tools, we show that subtle changes in mutations at codon 12 and 13 have a dramatic impact on tumor initiation in the pancreas, and that organoids carrying particular Kras alterations are differentially sensitive to targeted therapies.

The biochemical properties of individual KRAS mutant proteins have been well-characterized in cell-based models, and show specific differences in intrinsic or GAPmediated GTP hydrolysis and/or nucleotide exchange⁴. The phenotypic consequences for each mutation have been much more difficult to define. Recently, Winters et al used a multiplexed adeno-associated virus (AAV) approach to engineer endogenous KRAS mutations in the lung and pancreas of recipient mice ⁴⁰. These experiments concluded that, in multiple sensitized backgrounds, G12D, G12R, and G13R mutations readily induce tumor growth, while G12C is a comparatively weak transforming allele. In contrast, our data in the pancreas, KP organoids, and MEFs, suggest that G12C is quite a potent KRAS mutant, while G12R drives a less robust KRAS phenotype. There are notable differences between these studies, including the timing of KRAS activation, presence/absence of co-altered tumor suppressors, and tissue context, but there is no clear indication of what underlies the phenotypic differences observed. One important technical consideration that may have relevant biological consequences is that using CRISPR-based HDR to engineer Kras mutations, Winters et al, invariably introduced disruptive mutations in the second WT Kras allele, as we observed during the first iteration of targeting ES cells. Whereas, our Cre-driven models retains the expression of WT Kras. Indeed, in some contexts, WT KRAS acts as a tumor suppressor⁴¹ and can influence the types of KRAS mutations that occur following chemical carcinogenesis⁴². The role of WT KRAS protein is a poorly understood, but important question in cancer biology, as up to 50% of all KRAS mutant cancers show allelic imbalance involving amplification of the mutant gene, or loss of the wildtype copy⁴³. In this regard, our new mouse alleles offer a controlled setting to study the impact of WT Kras, by exploiting the silent mutations introduced the mutant alleles (Figure 1b) and selectively targeting the WT allele by CRISPR or RNAi-based strategies.

We noted striking differences among KRAS mutants in early transformation of the pancreatic epithelium, which persisted even following acute pancreatitis, as G12R mutants failed to progress through the acinar to ductal transition to form PanIN lesions. KRAS^{G12R} pancreata showed a significantly reduced frequency of DCLK1+ progenitors that are linked to pancreatic cell transformation²⁸ but it is not possible to tell whether this is a cause or consequence of the stalled transformation. G12R lesions appear to have less ERK42/44 phosphorylation, but this is most likely linked to the lack of ductal cells where pERK is highest (Figure 2). Interestingly, Hobbs et al, recently revealed that KRASG12R mutant cells have reduced AKT/PI3K activation and micropinocytosis8. Both PI3K signaling and micropinocytosis have been directly linked to tumor initiation and/or progression in KRAS-driven pancreas cancer⁴⁴⁻⁴⁶, providing a potential mechanism for this atypical *in vivo* response. Indeed, we noted diminished AKT phosphorylation and lower PI3K/AKT/MTOR transcriptional signatures in G12R mutant organoids (Figure 4). It will be important to investigate the consequences of this altered signaling in models of later stage pancreatic cancer.

To explore the utility of the LSL-Kras^{MUT} strains as preclinical tools, we generated KP pancreatic organoids and systematically tested two different targeted therapies. The response of G13D mutants to EGFR inhibitors that we observed in pancreatic organoids (Figure 4) is consistent with retrospective clinical data in colorectal cancer^{6,7}, and two recent publications using cancer cell lines. Like these studies, our work shows that NF1 is an important regulator of MAPK signaling output in KRAS^{G13D} mutant cells, though the exact mechanism remains controversial^{33,34}. These observations parallel similar findings in BRAF mutant cells, where Rosen and colleagues identified distinct classes of oncogenic BRAF mutations based on their signaling dependencies³¹. Class III BRAF mutations, like KRASG13D mutations more frequently cooccur with mutations in additional MAPK pathway genes, and human cancers carrying these alterations are more sensitive to EGFR inhibition⁴⁷. Similarly, KRAS^{A146T} mutations commonly co-occur with MAPK pathway mutations in CRC, and Poulin et al recently showed using a similar Cre-conditional Kras approach, that A146T mutations are poorly transforming in the colon and pancreas, like G13D^{48,49}. Together, these mutations likely represent a distinct class of KRAS alterations that may be differentially vulnerable to existing clinical therapies.

Finally, we show that KP organoids carrying an endogenous KRAS^{G12C} mutation are sensitive to a recently described covalent G12C inhibitor, but that response to this drug can be bypassed by upstream EGFR signaling. Similar synergistic effects of G12C and EGFR inhibitors have been noted in human cancer cell lines^{36,38,39}, potentially due to reduced SOS-dependent GDP-GTP exchange, increasing the GDP-bound pool of KRAS^{G12C} and rendering it more vulnerable to covalent modification^{37,38,50}. It may also be possible that the presence of WT KRAS in these cells allows escape from targeted G12C inhibition, and that this activity is RTK dependent. Further work in genetically defined systems such as these will provide a complete picture of the signaling and phenotypic consequences of clinical G12C inhibitors, to further guide clinical application of these exciting small molecules.

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Together these data describe the development of three new broadly useful precision oncology models, and highlight unique downstream consequences of subtle and cancerrelevant changes in KRAS. The models faithfully represent the signaling dynamics and therapeutic response of human cancer cells and we expect they will serve as valuable immunocompetent pre-clinical tools to understand KRAS biology and develop more effective treatment strategies for KRAS-driven cancers.

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Author Contributions

MPZ designed and performed experiments, analyzed data and wrote the paper. DAC, SG, EMS, TH and JW performed experiments and/or analyzed data. LED designed, performed and supervised experiments, analyzed data, and wrote the paper.

Conflict of Interest Statement

LED is a scientific advisor for Mirimus Inc.

METHODS

Cloning

VP12 vector expressing spCas9-HF1 15 was codon optimized 16 and renamed HF1*. Sequences encoding Kras sgRNAs (Supplementary Table 2) were cloned into BbsI site of pX458, VP12-U6 and pXHF1* vectors. P53c sgRNA (Supplementary Table 2) was cloned into BsmBI site of Lenti-Cas9-Cre (LCC) vector. For shRNA cloning NF1 and Renilla 713 shRNAs (Supplementary Table 2) were cloned into Xhol/EcoRI site of SGEN vector 51.

ESC targeting

The p48 embryonic stem cell (ESC) line, derived previously as described somewhere else ¹⁴, was used to generate the new conditional LSL-Kras strains. ESC were cultured in KOSR + 2i media ⁵² on irradiated mouse embryonic fibloblast (MEF) feeder layers. 2x10⁵ cells were co-transfected with 2ug of the Cas9/sgRNA vector PxHF1* and 4 ul of ssODN HDR template (20 uM) using a Lonza X-Unit Nucleofector with P3 buffer kit (Lonza #V4XP-3032). Four days following transfection, cells were plated at low density (500 cells) to enable clonal growth and the remaining culture was used to assess targeting efficiency from bulk population (see methods below). Clones were picked when they were visible without microscope. PCR amplification following digest to confirm template integration were carried out. Digested products were analyzed by QIAxcel (Qiagen). Positive clones were expanded and further validated by allele specific PCR and Sanger sequencing before send them to perform blastocyst injection.

Clone screening

Clones were picked and trypsinized. Half of the volume was mixed with 2x DNA lysis buffer (20 mM Tris, pH 8.8, 40 mM (NH4)2SO4, 20 mM MgCl2, 10%Triton X 100, proteinase K 800 ug/ml, β -ME) and incubated for 2 h at 55°C following 20 min at 95°C to inactivate the Proteinase K. The remaining half was resuspended in media and transfer to a 48 well plate already containing 500 ul of ESC media. The region of interest was amplified using 1ul of the crude gDNA lysis in 16ul volume using Promega 2X PCR master mix. HDR targeting was confirmed in each clone by digesting for 2 h half of the PCR product (8 ul) with the specific restriction enzyme for each integrated template (Figure 1b).

Genomic DNA isolation, and T7 assay

ESCs were lysed in genomic lysis buffer (10 mM Tris, pH 7.5, 10 mM EDTA, 0.5% SDS, and 400 μ g/ml proteinase K) for at least 2 h at 55 °C. After proteinase K heat inactivation at 95 °C for 15 min, 0.5 volume of 5

M NaCl was added, and samples were centrifuged for 10 min at 15,000 r.p.m. Supernatants were mixed with one volume of isopropanol, and DNA precipitates were washed in 70% EtOH before resuspension in 10 mM Tris, pH 8.0. Cas9-induced mutations were detected using the T7 endonuclease I 53. Briefly, the target region surrounding the expected mutation site was PCR amplified using Herculase II (600675, Agilent Technologies). PCR products were column-purified (Qiagen) and subjected to a series of melt–anneal temperature cycles with annealing temperatures gradually lowered in each successive cycle. T7 endonuclease I was then added to selectively digest heteroduplex DNA. Digest products were visualized on a 2.5% agarose gel.

DNA-library preparation and MiSeq

Deep sequencing was performed on Clones Cas9-only transfected or cotransfected alongside an HDR template and successfully having integrated it. Briefly, DNA-library preparation and sequencing reactions were conducted at GENEWIZ. A NEB NextUltra DNA Library Preparation kit was used according to the manufacturer's recommendations (Illumina). Adaptorligated DNA was indexed and enriched through limited-cycle PCR. The DNA library was validated with a TapeStation (Agilent) and was quantified with a Qubit 2.0 fluorometer. The DNA library was quantified through realtime PCR (Applied Biosystems). The DNA library was loaded on an Illumina MiSeq instrument according to the manufacturer's instructions (Illumina). Sequencing was performed with a 2 × 250bp paired-end configuration. Indel calling was performed using CRISPResso2.

Virus production

For virus production, HEK293T cells (ATCC CRL-3216) were plated in a 10 cm plate and transfected 12 h later (at 95% confluence) with a prepared mix in DMEM (with no supplements) containing 15 μ g of lentiviral backbone LCMCp53c (pLenti-U6-p53c-sgRNA-Cas9-p2A-Cre), 7.5 μ g of PAX2, 3.75 μ g of VSV-G, and 78 μ l of polyethylenimine (1 mg/ml). 36 h after transfection, the medium was replaced with target cell collection medium, and supernatants were harvested every 8–12 h up to 72 h after transfection.

Animal Studies

Production of mice and all treatments described were approved by the Institutional Animal Care and Use Committee (IACUC)at Weill Cornell Medicine, under protocol number 2014-0038. ES cell-derived mice were produced by blastocyst injection, and animals were either maintained on a mixed C57B6/129 background for experimental breeding or back-crossed to C57BL/6N mice. All LSL-Kras strains are available from Jackson Labs (G12C: B6N.129S4-Kras^{em1Ldow}/J (#033068); G12R: B6N.129S4-Kras^{em2Ldow}/J (#033316); G13D: B6N.129S4-Kras^{em3Ldow}/J (#033317). Progeny of both sexes were used for experiments and were genotyped for specific alleles (LSL-KrasG12D, LSL-KrasG12C, LSL-KrasG12R, LSL-KrasG13D, Ptf1/ p48-Cre, Rosa26-LSL-tdTomato, CAGS-LSL-RIK) using primers described in Supplementary Table 2 and protocols available at www.dowlab.org/ Protocols. Production of mice and all treatments described were approved by the IACUC at Weill Cornell Medicine under protocol number 2014-0038. To induce experimental pancreatitis 9-week-old mice were subjected to 8 intraperitoneal injections of caerulein (50µg/kg of body weight) once every 1 hour 54. Mice were monitored daily, and euthanized 20 days after the acute treatment. components of the mouse immune system do not fully mature until after approximately 4-6 weeks of age.

Mouse Embryonic Fibroblasts (MEFs)

KrasLSL-mut /LSL-tdTomato males were bred with C57BL/6N females. MEFs were derived from E12.5-E14.5 embryos following previously described protocol ⁵⁵. Cells were cultured and expanded for one passage in DMEM (Corning) supplemented with 10% (v/v) FBS, and frozen at passage 2.

MEF immortalization

MEFs were immortalized by using a vector encoding Cas9 and a p53c sgRNA, as well as Cre recombinase to be able to activate Kras. Cells were thawed and immediately transduced with viral supernatants (1:2) in the presence of polybrene (8 μ g/µl). Two days after transduction cells were selected in Nutlin-3 (10 µM). Stablished MEF cell lines expressing different Kras mutants (G12D, G12C, G12R and G13D) or KrasWT and Tp53 loss, were consequently used to perform RNAseq and western blot analysis. For protein experiments MEFs were starved overnight (2 % FBS DMEM medium) and then stimulated with EGF 20 ng/ml for 10 min (refs). All MEF data were obtained using at least 3 independent MEFs/genotype.

Fluorescence competitive proliferation assays

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MEFs were thawed and after one passage infected with adenovirus-Cre purchased from University of Iowa (5×10^7 plaque-forming units / 1×10^6 cells). One day after infection, the percentage of tdTomato-positive cells was measured by flow cytometry (Attune NxT Flow Cytometer, Thermo Scientific) and cells were mixed at define proportions with their respective parental cells. tdTomato fluorescence was then tracked every 5 days by flow cytometry.

Ras-GTP-Pull down

Ras-GTP levels were assessed by Active Ras Pull-Down and Detection Kit (Thermo Scientific, Cat#16117Y) using Raf-RBD fused to GST to bind active (GTP-bound) Ras. Protein lysates (500 µg) were incubated with 30 µL glutathione resin and GST protein binding domains for one hour at 4°C to capture active small GTPases according to the manufacturer's protocol. After washing, the bound GTPase was recovered by eluting the GST-fusion protein from the glutathione resin. The purified GTPase was detected by western blot using mouse monoclonal anti-KRAS provided by the Kit.

Murine Pancreatic Ductal Organoid Culture

Isolation of normal pancreatic ducts was done modifying previously described protocol 56. Briefly, pancreas was minced and washed in Hanks's Balanced Salt Solution (Corning), and then incubated for 30 min at 37°C with Collagenase V to release the ducts. After washing twice with DMEM/10% FBS media, ducts were resuspended in basal media [Advanced DMEM/F12 (Corning) containing 1% penicillin/streptomycin, 1% glutamine, 1.25 mM N-acetylcysteine (Sigma Aldrich A9165-SG) and B27 Supplement (Gibco)], and mixed 1:10 with factor reduced (GFR) Matrigel (BD Biosciences). Forty microliters of the resuspension was plated per well in a 48-well plate and placed in a 37°C incubator to polymerize for 10 minutes. To culture ductal pancreatic organoids the basal media described above was supplemented with 10 nM Gastrin (Sigma), 50 ng/ml EGF (Peprotech), 10% RSPO1conditioned media, 100 ng/ml Noggin (Peprotech), 100 ng/ml FGF10 (Peprotech) and 10 mM Nicotinamide (Sigma). Note: Culture freshly isolated organoids in pancreatic organoid media (POM) containing 10µM Rock inhibitor (Y2732) during 72-48 h. For subculture and maintenance, media were changed on organoids every two days and they were passaged 1:3 every 5 days. To passage, the growth media was removed and the Matrigel was resuspended in cold basal media and transferred to a 15-mL Falcon tube. Organoids were mechanically disassociated using a P1000 and pipetting 40 times. Five milliliters of cold PBS were added to the tube and cells were then centrifuged at 1,200 rpm for 5 minutes and the supernatant was aspirated. Cells were then resuspended in GFR Matrigel and replated as above. For freezing, after spinning the cells were resuspended in complete containing 10% FBS and 10% DMSO and stored in liquid nitrogen indefinitely.

Organoid Transduction

To generate KP organoids (Krasmut/Tp53 loss), normal pancreatic organoids were cultured in transduction media [POM containing CHIR99021 (5 μ M) and Y-27632 (10 μ M)] for 2 days prior to transduction. Single-cell suspensions were produced by dissociating organoids with TrypLE Express (Invitrogen#12604) for 5 minutes at 37°C. After trypsinization, cell clusters were resuspended in 400 μ l of transduction media containing concentrated lentiviral particles in the presence of polybrene (8 μ g/ μ l) and transferred into a 48-well culture plate. The plate was centrifuged at 600 x g at 32°C for 60 minutes, followed by another 4-hour incubation at 37°C. Cell clusters were spun down and plated in Matrigel.

Organoid drug treatment

Organoids were plated in 120 μ L Matrigel (3 x 40 μ L droplets) in one 12-well plate and cultured in basal media with either DMSO or Gefitinib (1 μ M) or ARS-1620 (1 μ M) or Gef/ARS combined. Organoids were passaged 1:3 every 72 h and then cultured again in DMSO, Gefitinib, ARS-1620 or Gef/ARS combination.

EdU Flow Citometry and Imaging in Organoids

Organoid EdU flow cytometry was performed using the Click-iT Plus EdU Alexa Fluor 647 Flow Cytometry Assay Kit (Thermo Fisher Scientific, # C10634). Pancreatic organoids were first incubated with 10 μ M EdU for 4 hours at 37°C. One well of a 12-well plate was broken up by pipetting vigorously 50 times in 1 mL PBS, then diluted in 5 mL of PBS. Cells were pelleted at 1,100 rpm x 4 minutes at 4°C, then resuspended in 50 μ L TrypLE and incubated at 37°C for 5 minutes. Five milliliters of PBS were then added to inactivate the TrypLE, and cells were pelleted. Cells were resuspended in 250 μ L of 1% BSA in PBS, transferred to a 1.7-mL tube, and then pelleted at 3,000 rpm x 4 minutes. Cells were then resuspended in 100 μ L Click-iT fixative, and processed as instructed in the Click-iT Plus EdU protocol (starting with Step 4.3). Wash and reaction volumes were 250 µL. For imaging, organoids were stained as described previously ⁵⁷. Images were acquired using Zeiss LSM 880 laser scanning confocal microscope, and Zeiss image acquiring and processing software. Images were processed using FIJI (Image J) and Photoshop CS5 software (Adobe Systems, San Jose, CA, USA).

RNA isolation, cDNA synthesis, and qPCR

To isolate RNA from MEFs and pancreatic organoids we used TRIzol (Thermo Fisher Scientific, #15596018) according to the manufacturer's instructions, and contaminating DNA was removed by DNase treatment for 10 minutes and column purification (Qiagen RNeasy #74106). Pancreas tissue portion for RNA purification was consistently collected from the tail of the organ and immediately cut into smaller pieces and immerse in RNAlater stabilization solution (Thermo Fisher) and incubate at 4°C overnight before storing the sample at -80°C until RNA extraction was performed. Samples were homogenized using a Tissue Master 125 (Omni) and RNA purified using the RNAeasy Kit (Qiagen).

RNA sequencing

Total RNA was isolated using Trizol, DNAse treated and purified using the RNeasy mini kit (Qiagen, Hilden, Germany). Following RNA isolation, total RNA integrity was checked using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA concentrations were measured using the NanoDrop system (Thermo Fisher Scientific, Inc., Waltham, MA). Preparation of RNA sample library and RNAseq were performed by the Genomics Core Laboratory at Weill Cornell Medicine. Messenger RNA was prepared using TruSeq Stranded mRNA Sample Library Preparation kit (Illumina, San Diego, CA), according to the manufacturer's instructions. The normalized cDNA libraries were pooled and sequenced on Illumina NextSeq500 sequencer with single-end 75 cycles.

RNAseq analysis

Transcript abundance estimates were performed using Kallisto ⁵⁹, aligned to the same (GRCm38) reference genome. Kallisto transcript count data for each sample was concatenated, and transcript per million (TPM) data was reported for each gene after mapping gene symbols to ensemble IDs using R packages, "tximport", tximportData", "ensembldb", and "EnsDb.Mmusculus. v79". Differential gene expression was estimated using DESeq2 ⁶⁰. For data visualization and gene ranking, log fold changes were adjusted using IfcShrink in DESeq2, to minimize the effect size of poorly expressed genes. GSEA analysis (v3.0) was performed on pre-ranked gene sets from differential expression between control and treated groups. We used R (v3.6.1) and R Studio (v1.2.1335) to create all visualizations, perform hierarchical clustering and principal component analysis. Volcano plots, heatmaps and other visualizations were produced using the software packages:

Enhanced Volcano (https://bioconductor.org/packages/devel/bioc/html/ EnhancedVolcano.html)

pheatmap (https://cran.r-project.org/web/packages/pheatmap/index.html) ggplot2 (https://cran.r-project.org/web/packages/ggplot2/index.html)

Whole exome sequencing

Each gDNA sample based on Qubit quantification are mechanically fragmented on an Covaris E220 focused ultrasonicator (Covaris, Woburn, MA, USA). Two hundred ng of sheared gDNA were used to perform end repair, A-tailing and adapter ligation with Agilent SureSelect XT (Agilent Technologies, Santa Clara, CA) library preparation kit following the manufacturer instructions. Then, the libraries are captured using Agilent SureSelectXT Mouse All Exon probes, and amplified. The quality and quantities of the final libraries were checked by Agilent 2100 Bioanalyzer and Invitrogen Qubit 4.0 Fluorometer (Thermo Fisher, Waltham, MA), libraries are pooled at 8 samples per lane and sequenced on an Illumina HiSeq 4000 sequencer (Illumina Inc, San Diego, CA) at PE 2×100 cycles. Copy number alterations were identified and plotted using cnvkit (v0.9.6) and single nucleotide variant called using MuTect2.

Protein Analysis

Pancreatic organoids were grown in 120 μ L of Matrigel in one well of a 12-well dish. Organoids were then recovered from the Matrigel using Cell Recovery Solution. Organoid pellets were lysed in 30 μ L RIPA buffer. Antibodies used for Western blot analysis were: anti-actin-HRP (Abcam #ab49900), anti- α -Tubulin (Millipore Sigma #CP06), anti-pERK 44/42 (Cell Signaling Technology #4370), anti-ERK 44/42 (Cell Signaling Technology #9107), anti-pAKT (ser 473) (Cell Signaling Technology #4060), anti-AKT (Cell Signaling Technology #4691), anti-NF1 (Cell Signaling Technology #14623).

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Immunofluorescence and Immunohistochemistry

Tissue, fixed in freshly prepared 4% paraformaldehyde for 24 hours, was embedded in paraffin, and sectioned by IDEXX RADIL. Sections were rehydrated and unmasked (antigen retrieval) by heat treatment for 10 minutes in a pressure cooker in 10 mM Tris/1 mM EDTA buffer (pH 9) containing 0.05% Tween 20. For immunohistochemistry, sections were treated with 3% H2O2 for 10 min and blocked in TBS/0.1% Triton X-100 containing 1% BSA. For immunofluorescence, sections were not treated with peroxidase. Primary antibodies, incubated at 4°C overnight in blocking buffer, were: rabbit anti-Ck19 (1:400, Abcam #ab133496), rabbit anti-Dclk1 (1:400, Abcam #109029), goat anti-CPA1 (1:400, R&D Systems AF2765), rabbit anti-αSMA (1:400, Abcam #ab5694), rabbit anti-pERK 44/42 (1:1000, Cell Signaling Technology #9101), rabbit anti-Sox9 (1:1,000, Millipore #AB5535), rabbit anti-tRFP (1:2000, Evogren #AB233). For immunohistochemistry, sections were incubated with anti-rabbit ImmPRESS HRP-conjugated secondary antibodies (Vector Laboratories, #MP7401) and chromagen development was performed using ImmPact DAB (Vector Laboratories, #SK4105). Stained slides were counterstained with Harris' hematoxylin and mounted with Mowiol mounting media. For immunofluorescent stains, secondary antibodies were applied in TBS for 1 h at room temperature in the dark, washed twice with TBS, counterstained for 5 min with DAPI and mounted in ProLong Gold (Life Technologies, #P36930). Secondary antibodies used were: donkey anti-rabbit 594 (1:500, Invitrogen #A21207), donkey anti-goat 488 (1:500, Invitrogen #A11055). Masson's Trichrome stainings were performed by IDEXX Radil. Images of fluorescent and IHC stained sections were acquired on a Zeiss Axioscope Imager (chromogenic stains), Nikon Eclipse T1microscope (IF stains). Raw.tif files were processed using FIJI (Image J) and/or Photoshop (Adobe Systems, San Jose, CA, USA) to create stacks, adjust levels and/or apply false colouring.

Data Availability

Raw exomeSeq and RNAseq data have been deposited in the sequence read archive (SRA) under accession PRJNA578549.

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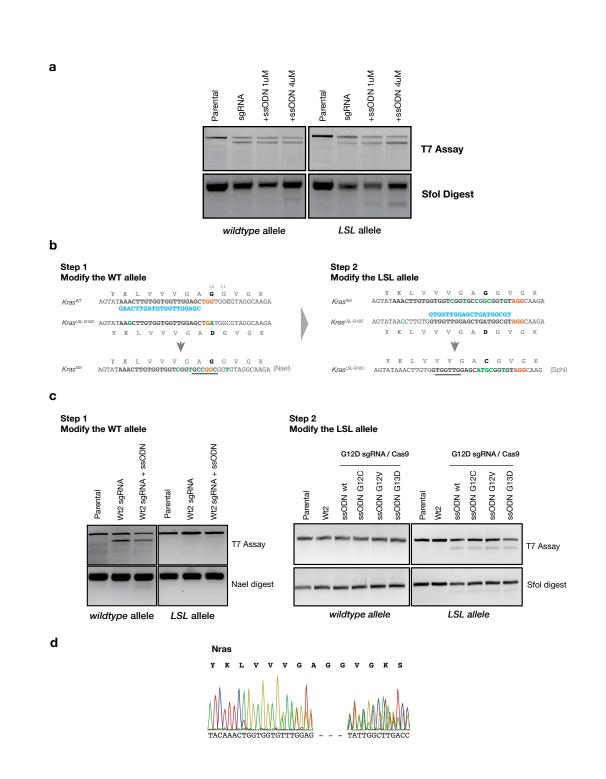
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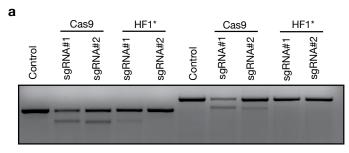
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Supplementary Figure 1. Summary of attempts to generate allele-specific HDR knock-in in ESCs. a. T7 endonuclease assay and Sfol digest of Kras allele-specific PCR products from bulk transfected ESC population 4 days after delivery of Cas9, KrasG12D sgRNA and different concentrations of ssODN, as noted. All HDR targeting experiments were performed with 4uM ssODN. **b.** Schematic showing the strategy followed to introduce silent mutations in the WT allele (Step 1) to be able to selectively target the LSL allele in a second round of targeting (Step 2). **c.** T7 endonuclease assays and Nael (left panel) / Sfol (right panel) digests showing selective targeting of the WT allele (left) and subsequent targeting of the LSL allele (right). **d.** Example Sanger sequencing chromatogram showing disruptions to Nras gene following targeting with Kras WT2 sgRNA.

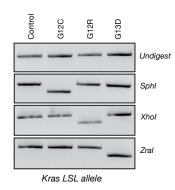
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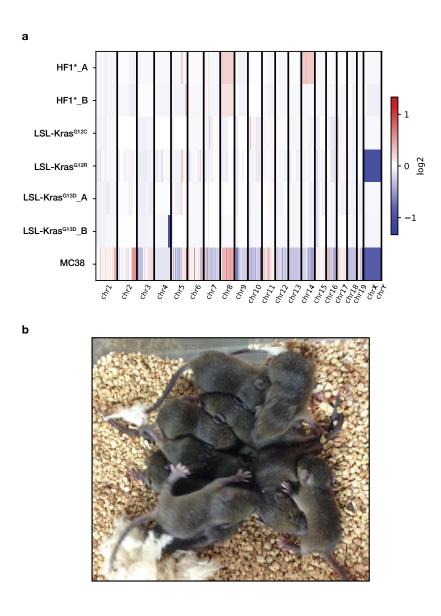
LSL-Kras allele

WT-Kras allele

b



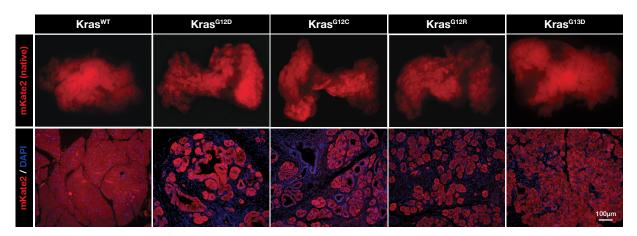
Supplementary Figure 2. Validation of high-fidelity targeting and resulting ESC clones used for mouse production. a. T7 endonuclease assay performed in each Kras allele-specific PCR product amplified from ESC bulk population 4 days after co-transfecting either with regular SpCas9 (PX458) or SpCas9-HFc using two different KrasG12D sgRNAs. b. Restriction digests of LSL-allele specific PCR products from ESC clones carrying Kras-LSLG12C, Kras-LSLG12R or Kras-LSLG13D knock-in mutations.



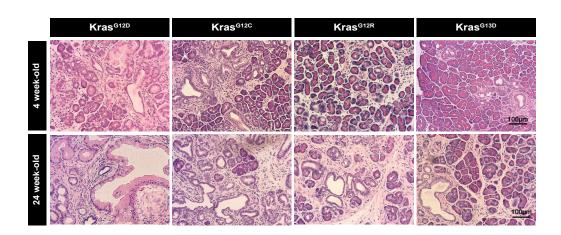
Supplementary Figure 3. Copy number analysis in HDR-targeted ESC clones used for mouse generation. a. Graph displaying copy number variation in 2 HF1*-only transfected clones, 4 HDR-targeted clones, and the murine cell line MC38 as reference for CNVs. b. Example of high contribution chimeras derived from blastocyst injection of ESC genetically modified carrying the LSL-KrasG12R allele.

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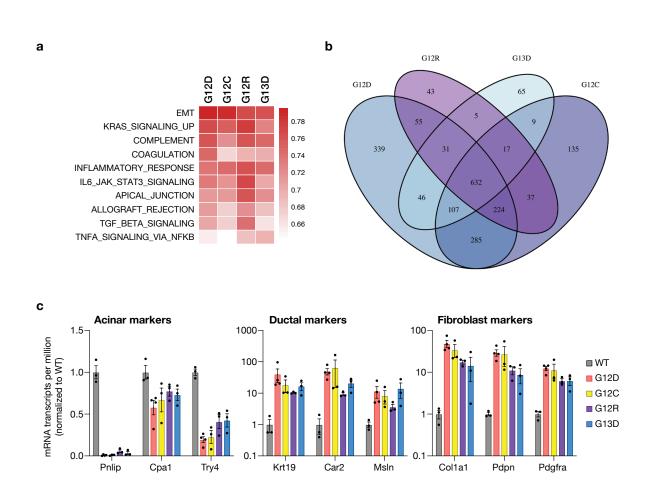




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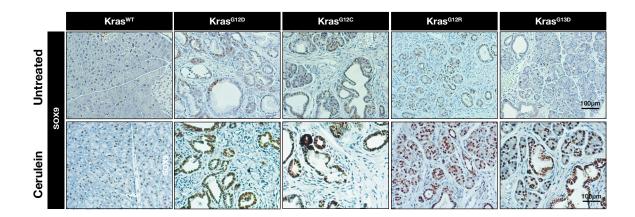


Supplementary Figure 4. Analysis of Kras mutant whole pancreas. a. Wholemount fluorescence (upper) and mKate2 immunofluorescence staining (lower) from pancreata of 12-week-old mice. b. Hematoxylin & eosin stained-sections of 4-week-old (top panel) and 24-week-old (low panel) pancreas from each genotype, as indicated.



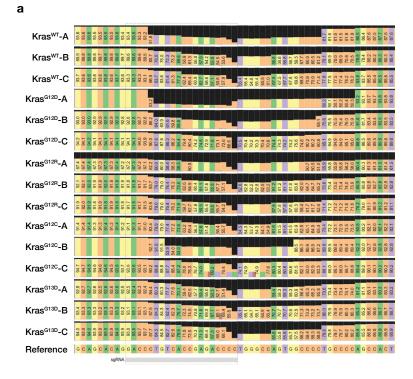
Supplementary Figure 5. Transcriptome analysis of Kras mutant whole pancreas. a. Gene set enrichment analysis summary displaying the top 10 enriched pathways in KrasG12D pancreata, compared to WT. **b.** Venn diagram showing the number of common differentially expressed genes (log2FC > 2, adjusted p-value < 0.01) among the 4 different Kras mutants (G12D, G12C, G12R and G13D), compared to WT pancreas **c.** mRNA expression (transcripts per million estimates) of acinar, ductal and fibroblast markers. RNAseq was performed in 4-week-old mouse pancreata (n=3 mice per genotype).

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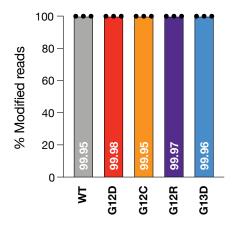


Supplementary Figure 6. Sox9 is induced in Kras mutant pancreatic epithelium. Sox9 immunohistochemical staining performed in 12-weeks-old pancreas from untreated (top panel) or cerulein-treated mice of each Kras genotype, as indicated.

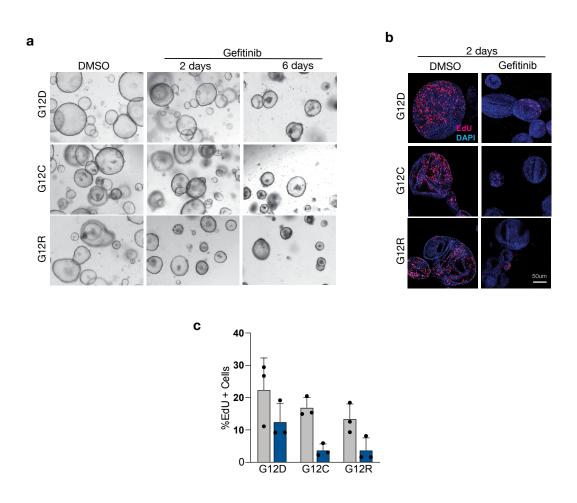
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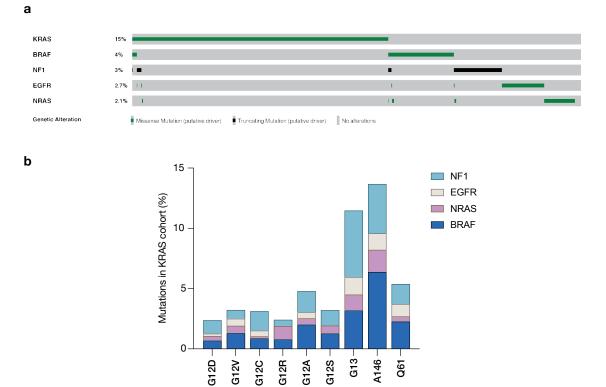


Supplementary Figure 7. CRISPResso2 analysis of targeted deep sequencing of the Trp53 sgRNA target site in KP organoids. a. Plot of the modified-nucleotide percentage achieved after targeting pancreatic organoids with a Tp53 sgRNA. **b.** Graph bar displaying the percentage of mutant Trp53 reads within the targeted Tp53 region.



Supplementary Figure 8. Impact of EGFR inhibition in G12R and G12C pancreatic organoids. a. Brightfield images from G12C and G12R KP organoids treated with DMSO or 1 μ M Geftinib for 2 and 6 days. Immunofluorescent images (b) and EdU flow cytometry (c) from G12C and G13D KP organoids treated with DMSO or Gefitinib for 2 days. Proliferating organoids are marked by EdU (red). Bar graph represents n=3 independent biological replicates per genotype +/- SD.

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Supplementary Figure 9. Co-mutation frequency of MAPK pathway genes in human cancer a. Oncoprint from Genomic Evidence Neoplasia Information Exchange (GENIE) dataset, highlighting minimal overlap of mutations within MAPK pathway genes across all cancer types. **b.** Expanded detail of co-mutation data for NF1, EGFR, NRAS, and BRAF in KRAS mutant cancers summarized in Figure 4g. In this cases, sub-divided by specific codon 12 substitutions. Data obtained from GENIE dataset.

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Positive clones / # Screened
1/96
2/144
1/96
1/48
3/96
2/96

Table 1. Clone identification frequency

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Supplementary Table 1. Mutations identified in ESC clones distinct from parental cells

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Cm2683n.114725-Tp.Ser477bamThen2161.1250C-Tp.Ser471bamThen2161.1260C-Tp.Val950ValmyZP445C.644-40C-Ap.Val271fafraGm0304C.128-40A-Cp.Val271fafraGm0304C.128-40A-CmfraGm1477C.880-477-S0p.Val271fafraGm1474C.465-17-S0mfraGm1474C.465-17-S0mfraGm1474C.465-17-S0mfraGm1474C.465-17-S0mfraGm1474C.4695-GAp.Au1567fmmGm1474C.4695-GAp.Au1567fmmGm1474C.4695-GAp.Au1567fmmGm1475C.4696-GAp.Au1567fmmGm1485C.531-1563-GD-TmmGm10585D.501G-TmmSpr30C.256-Ap.Lau477fLeumSpr30C.256-Ap.Lau477fLeumSpr30C.256-Ap.Lau477fLeumSpr30C.250-GAp.Lau477fLeumSpr30C.250-GAp.Lau477fLeumSpr30C.250-GAp.Lau59fmmSpr30C.250-GAp.Lau59fmmSpr30C.250-GAp.Lau59fmmSpr30C.230-GAp.Lau59fmmSpr30C.230-GAp.Lau59fmmMarinC.230-GAp.Ma9314mSpr30C.230-GAp.Ma9314mMarinC.	missense_variant	0.211 (24/116)	-						
Pichc.1280C>Tp.Sar430*p.Sar440*p.Sar40*Cachd1c.2880C>Ap.Val950ValminCachd1c.2880C>Ap.Val950ValminPip210c.811_9160*p.Val9718minGm1127c.804-40C>Ap.Ang1687pminGm1127c.804-77-GminGm1127c.804-77-GminGm1127c.8040-57minGm1124c.1240-56C>TminFam1680c.4840-5CminGm1124c.4805-CminCharlc.68405C-TminCharlc.68405C-TminCharlc.68405C-TminGm1055c.1240-56C-TminGm1065n.5016-TminGm1065n.5016-TminGm107c.8252-S2-ManGminGm1085n.5016-TminSpc30c.2526-S4-ManGminGm1085n.5016-TminSpc30c.2526-S4-ManGminSpc30c.2526-S4-ManGminMp10c.1473G-CminAg8b5c.104-173G-Ap.Ag92A8nMp11c.286C>TminAg9210c.232C-TminMp211c.286C>TminAg9211c.286C>TminAg9211c.286C>TminAg9211c.286C>TminAg9211c.286C>TminAg9211c.286C>TminAg9211c.286C>TminMp20c.35712-54067minMp3	ntron_variant	0.211 (24/116)							
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Gm 1127c. 800-7T-Gp. Qm 168TpmChaffac. 3375G-AmChaffac. 23375G-AmGm 1474c. 4485-Tp. Qm 1584KIc. 2484-163-GmitKIc. 2484-163-GmitKIc. 2486-GAp. Alaf 1586ThrmitCharlac. 2486-GAp. Alaf 1586ThrmitCharlac. 24832-SGmitCallac. 8843816-ATmCallac. 8843816-ATmCallac. 8843816-ATmCallac. 8843816-ATmSyrpic. 258-GAp. Ags 9AanSyrpic. 258-GAp. Ags 9AanCallac. 258-GAp. Ags 9AanSyrpic. 258-GAp. Jaur 770-GAMithic. 252-GAp. Jaur 770-GAMithic. 252-GAp. Jaur 770-GAMithic. 1417G-GAmMagbasc. 1417G-GAmMarghatc. 1523-GAp. Jaur 770-GAMarghatc. 1523-GAp. Jaur 770-GAMarghatc. 1523-GAp. Marg 771Marghatc. 2586-GTmMarghatc. 1626-GCmMarghatc. 1627-GCmMarghatc. 1627-GCmMarghatc. 1627-GCmMarghatc. 1627-GCmMarghatc. 1627-GCmMarghatc. 1627-GCmMarghatc. 1627-GCmMarghatc. 1627-GCmMarghatc. 1626-GC<	ntron_variant	0.096 (16/171)							
ChaftaqC.4405.TC.yast5PhemiFam168aC.4405.CAP.Gyst5PhemiKitC.2405.CAP.Gyst5PhemiChrantoC.4405.CAP.Galy21.7ArgmiChrantoC.4405.CAP.Galy21.7ArgmiChrantoC.252.SCAP.Galy21.7ArgmiPicxadC.252.SCAP.Galy21.7ArgmiChrantoC.252.SCAP.Galy21.7ArgmiSyrp3C.25G.AAP.Asp0.AanmiSyrp3C.25G.AAP.Asp0.AanmiB20355F06HikC.244.170.CAP.Galy21.2MamiB20355F06HikC.244.170.CAP.Galy21.2MamiB20355F06HikC.244.170.CAP.Leu.7F1.2MamiB20355F06HikC.244.170.CAP.Leu.7F1.2MamiB20355F06HikC.242.170.CAP.Leu.7F1.2MamiB2035F071C.142.0CAP.Leu.7F1.2MamiMarbi2C.152.0CAP.MeBVaImiMarbi2C.228.CATmimiMarbi2C.236.5ATP.MeBVAImiMarbi2C.325.5AP.Pr0313SermiGrid470N.152.2A3.9A406CAAP.MeBVAImiMarbi2C.325.5AP.Pr0313SermiGrid470N.152.2A3.9A406CAAP.MeBVAImiGrid520C.787.5AP.Pr0313SermiGrid470N.152.2A3.9A406CAAP.MeBVAImiGrid470C.325.5CTP.Pr0318ArmiGrid470C.325.5CTP.MeBSL1mi <tr< td=""><td>ntron_variant</td><td>0.096 (16/171)</td><td></td><td></td><td>-</td><td>-</td><td></td><td></td><td>-</td></tr<>	ntron_variant	0.096 (16/171)			-	-			-
Gan1474c.449s-140C-3Gp.Qsp15PheminFam168ac.449s-140C-3GintKitc.1240-450C-7p.Ala156ThrintChra10c.8493C-Gp.Ala156ThrintCall2n.849431G-TintCall2n.849431G-TintCall2n.849431G-TintCall2n.849431G-TintCall2n.849431G-TintCall2n.849431G-TintCall2n.849506C-Tp.Asp9AanintCall2n.849506C-Tp.Asp9AanintB2035970981kc.2440-7Ap.Sar28597intB2035970981kc.2440-7Ap.Sar28597intB2035970981kc.14173G-Cp.Asp32AppintAp2baCc.13C-Ap.Asp32AppintAp2baCc.13C-Ap.Asp32AppintAp2baCc.2278C-Tp.Asp32AppintAdryap11c.2626C-Tp.Asp32AppintAdryap11c.2626C-Tp.Met71intAdryap11c.2626C-Tp.Met71intAdryap11c.2626C-Tp.Met71intAdryap11c.2626C-Tp.Pro813SerintAdryap11c.2626C-Tp.Met71intAdryap1c.1524dupGintintAdryap1c.1524dupGp.Met71intAdryap1c.1524dupGp.Met71intAdryap1c.1524dupGp.Pro813LanintAdryap1c.2530-Cp.Pro813LanintAdryap2 <td>missense_variant</td> <td>0.085 (9/107)</td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> <td>-</td>	missense_variant	0.085 (9/107)	-	-	-	-	-	-	-
Fam Ban Kitc. 1240-83C-37Im My373bc. 1240-83C-37Im M M CIChmatOc. 4496G-Cp. Dity217ArgMatChmatOc. 4496G-Cp. Dity217ArgMatPicx3c. 1252S-GMatMatPicx3c. 1253G-SAMatMatCiflen. 8934596G/ShC. 454G-GMatSigr3c. 25G-AP. Asp9AsnMatB20359F08HKC. 24-10G-CMatB20359F08HKC. 24-10G-CMatB20359F08HKC. 24-10G-CMatB20359F08HKC. 24-10G-CMatB20359F08HKC. 24-10G-CMatB20359F08HKC. 24-10G-CMatB20359F08HKC. 24-10G-CMatB20359F08HKC. 24-10G-CMatB20359F08HKC. 24-10G-CMatB20359F08HKC. 24-10G-CMatMph9C. 1252G-AP. Jag-2024UDGAATMatMat210C. 226C-TMatMat310C. 226C-TMatMat310C. 226C-TMatMat310C. 226C-TMatMat310C. 226C-TMatMat310C. 226C-TMatMat310C. 226C-TMatMat310C. 226C-TMatMat310C. 226C-TMatMat310C. 127C-TP. MatMat310C. 226C-TMatMat310C. 127C-TP. MatMat310C. 127C-TP. MatMat310C. 127C-TP. MatMat310<	downstream_gene_variant	0.085 (9/107)	-	-	-	-	-		-
KitC. 440960-AP.Alat 506ThrmidYpat 3bC. 440960-Ap.Gly217ArgmidArt1C. 2502-C6P.Gly217ArgmidCell 2n. 69045A'CInmidGmi0085n. 5016-S'TmidmidSycp 3C. 2505-AP.Asp9AanmidSycp 3C. 2505-AP.Asp9AanmidSycp 3C. 2505-AP.Asp9AanmidSycp 3C. 2505-AP.Asp9AanmidSycp 3C. 2514-GACP.Lau/SturmidSycp 3C. 104-1790-SAP.Mar9AanmidSycp 3C. 104-1790-SAP.Asp93CanmidMayb 0C. 1437G-CP.Asp93CanmidMar1C. 132-SAP.Asp93CanmidMar2C. 132-SAP.Asp93CanmidMar5C. 142-SC-TmidmidMar5C. 2228-C-TmidmidMar5C. 142-SAP.VaBValmidMar1C. 2228-C-TmidmidMar1C. 118-27-UPGMidmidGmit 32C. 362-AP.Met 71midGmit 32C. 362-AP.Met 31midGmit 32C. 362-AP.Met 31midGmit 32C. 362-AP.Met 31midGmit 32C. 362-AP.Met 32midGmit 34C. 362-AP.Met 32midGmit 34C. 362-AP.Met 32midGmit 34C. 362-AP.Met 32midGmit 34C. 362-A <t< td=""><td>missense_variant ntron_variant</td><td></td><td>0.933 (74/79) 0.594 (28/46)</td><td></td><td></td><td></td><td></td><td></td><td></td></t<>	missense_variant ntron_variant		0.933 (74/79) 0.594 (28/46)						
Visit80c. 6.486S-CAp. Gly/217ArgmitChma10c. 2532C-SGp. Gly/217ArgmitPicx30c. 121503G-TmitGhl2n. 6394581G-TinGm1085n. 501G-Tp. Asp3AsnmitSyr030c. 250-SAp. Asp3AsnmitB230369708114c. 524-160CinmitB230369708114c. 524-160Cj. Asp3Asnj. Asp3AsnSur1370c. 524-61j. Asp3Asnj. Asp3AsnApB05c. 14173CS-Ap. Leu/371EmitApB25c. 1423GS-Ap. Leu/371EmitAp252c. 1523G>Ap. Leu/371EmitAdvap110c. 268-67Cj. Tarvi 3-4-vi<	ntron_variant		0.545 (19/35)			-			
Artic. 2523C-Gim in cnitim in cnitPicxd3c. 8124.1583C-Tin in cnitCnit2n. 834481C-Tin 	missense_variant		0.508 (82/161)		-				-
Pickalc.812.1630.S.TindCaff2n.834.861.S-TnnSipC3c.250.S.Ap.Asp.BAnnnSipC3c.250.S.Ap.Asp.BAnnnB2030597081Nc.28.41.10CintB2030597081Nc.28.41.10CintB2030597081Nc.28.41.10CintB2030597081Nc.28.41.10CintApBb5c.104.179G.Ap.Leu4791.euMy16c.130.S.Ap.Leu4791.euMy170c.130.S.Ap.Leu4791.euMap22c.152.3G.S.Ap.Jay50811Marp12c.122.8C.S.Tp.Map232.40Marp23c.2228.C.Tp.Map32.40My15c.2228.C.Tp.Map32.40My16c.2305.13G.S-Tp.Mat13Cafta.S.Ap.Mat17intGm1524n.182.40.0Gp.Mat17Marp1c.153.27.40.0Gp.Pro5138.erGm1637c.737.S.Ap.Mat17Gm1647c.238.63.G.Tp.Pro631.80Gm1678c.368.53.63.CTp.Pro631.80Gm1678c.368.54.64.0GTmintGm1679c.368.54.64.0GTmintGm1679c.368.63.64.64.0GTmintGm1679c.368.54.64.0GTmintGm1679c.368.54.64.0GTmintGm1679c.368.54.64.0GTmintGm1679c.368.54.64.0GTmintGm1679c.368.54.64.0GTmintGm1679c.368.54.64.0GTmintGm1679c.368.54.64.0GTmintGm1679c.368.54.64.0GTmin	missense_variant	-	0.5 (9/18)	-	-	-		-	-
Calf2n.80483fG-TmmGm10855n.5016>TmmStpG33C.250.Ap.AgpAnmmChpt1n.8449690C>TmmB20385PGNRikC.244.82.94msGmmB20385PGNRikC.244.11deCmmApBb5c.104.179G-Ap.Lau/471LauspApBb5c.104.179G-Ap.Lau/471LauspApBb5c.104.179G-Ap.Lau/471LauspApBb5c.104.179G-Ap.Lau/471LauspApBb5c.104.202p.Lau/471LauspApb20c.132.05-Ap.Lau/670LaummAdx3pa1r1c.2566-GTmmmmAdx3pa1r1c.2566-GTmmmmMm53c.2280-GTp.Ap32AspspStr321c.2280-GTp.MeR11emmAdx1m2c.1416-Ap.MeR11emmAdx1m3c.1523-53-9delCAAAp.Pro313SermmGr143c.1523-53-9delCAAAp.Pro313SermmGr143c.1523-Cp.Pro313SermmGr143c.1523-Gp.Ap4989ValmmGr143c.1523-Gp.Ap4989ValmmGr143c.1523-Gp.Ap498PValmmGr144c.363-GTp.Ap498PValmmGr145c.363-GTp.Ap498PValmmGr145c.1523-GTp.Ap498PValmmGr145c.363-GTp.Ap494PValmmGr145c.363-GTp.Ap494PValmmGr145c.363-GTp.Ap494PValmmGr145 <td>downstream_gene_variant</td> <td></td> <td>0.5 (9/18)</td> <td>-</td> <td>-</td> <td>-</td> <td></td> <td></td> <td>-</td>	downstream_gene_variant		0.5 (9/18)	-	-	-			-
Gm1085n.5010-Tp.Asp3AnnmmSycp3c.250-Ap.Asp4AnnmmB2003597081Kc.524-1104inthB2003597081Kc.524-1104inthB2003597081Kc.524-1104mApb5c.144706-Ap.Ser28567syTrav13-4v17c.1523-Ap.Leu479LeusyArb242c.1523-GAp.Jar9681mArb242c.1523-GAp.Jar9681mMcm5c.1286-GTp.Jar9681syMrag24c.2286-GTp.Val892ApsySi3211c.2286-GTp.Val892ApsySi3211c.2286-GTp.Val892ApsySi3211c.2286-GTp.Val892ApsySi3211c.2286-GTp.Val892AsySi3211c.2375-GTp.Val892AsySi3211c.1512-GTp.Val892AsySi3211c.1512-GTp.Pr0313SermGm1676n.860_681-GGTmGm1676n.860_681-GGTmGm1677c.2636-ATp.Pr031SermGm1678c.2635-ATp.Pr031SermGm1678c.2635-ATp.Pr034BanmGm16340n.802-38986-STmmGm16340c.363-GTp.Dig4894AmGm16340c.363-GTp.Dig483AmGm16340c.1644GC-GmmGm16340c.1644GC-GmmGm16340c.1644GC-GmmGm16340c.113-GCp.Dig387Am	intron_variant intragenic_variant	-	0.494 (46/92) 0.487 (75/155)		-	-		-	-
Sycp3c.250-Ap.Asp3Aenp.Asp3AenmmChp11n.8459600C>TintB20359F08HKc.524-0.52-48msGintB20359F08HKc.524-0.52-48msGintMy10c.140-179G-AintMy10c.143C>Ap.LeuSMetmmAp2b2c.13C>Ap.LeuSMetmmAlp2b2c.13C>Ap.LeuSMetmmAlp2b2c.13C>Ap.Jac932AspmsAdryap11c.268-G>C>Tp.Ms12MatmsAdryap11c.228-G>TmsmsSt021c.228-G>TmsmsAdryap11c.228-G>TmsmsAdryap11c.246-Ap.MetA'IIemsAdryap1c.326-STp.MetA'IIemsAdryap1c.141-G>Ap.Pro813SermsAdryap1c.132-S3-408C/AAp.Pro813SermsAdryap1c.337-STp.Pro81SermsGm1552c.152T>Cp.Pro81SermsGm1552c.152T>Cp.Pro83AanmsCapin2c.388-CTp.Met71staDp1c.2883C>Tp.Met8AmsCapin2c.182C>Gp.Cry85TsTmsMy402c.193C>Gp.Agr942MamsAdriac.194C>Gp.Agr942MamsAdriac.194C>Tp.Agr942MamsMy402c.193C>Gp.Agr942MamsMy403c.2883C>TmsmsMy404c.382C>Tp.Agr942MamsMy405c.193C>G	non_coding_transcript_exon_variant		0.487 (75/155)			-			
B203059089NKc5.24:1040CIntB20305908NKc5.24:11040CIntMyh0c1.3473-CPLBU/79LBUMyhMyh0c1.3473-CPLBU/79LBUMyhMyh1c1.3473-CPLBU/79LBUMyhMyh2c1.3523-CAPLBU/89LHSMyhAp2b2c1.5230-CAPLAUSMEHIntAdvjap11c.2696-CS-TPA\$9323-APSMyhMyn5c.22780-CTPA\$9323-APSMyhMyh2c.22780-CTPA\$9323-APSMyhMyh2c.22780-CTPA\$9323-APSMyhMyh2c.22780-CTPA\$9323-APSMyhMyh2c.246-CAPA\$9323-APSMyhGm5147n.1524-ApOMyhMyhGm5147n.1524-ApOMyhMyhGm4147n.1524-ApOMyhMyhGm4147n.1524-ApOMyhMyhGm4147n.1524-ApOPhe70313-SFMyhGm4147n.1524-ApOMyhMyhGm1457n.1820-APOPhe70313-SFMyhGm14540c.363-CTP.Ph08518-GTMyhGm14540c.363-CTP.Ph08518-GTMyhGm14540c.363-CTP.Ph08518-GTMyhGm14540c.363-CTP.Ph08518-GTMyhGm14540c.363-CTP.Ph08518-GTMyhGm14540c.363-CTP.Ph08518-GTMyhGm14540c.363-CTP.Ph08518-GTMyhGm14540c.363-CTP.Ph08518-GTMyhGm14540c.363-C	missense_variant		0.438 (19/42)		-	-			
B23059050Rikc.104.179G-AmmAp8b5c.104.179G-Ap.Leu/APILeusryShT2m3c.443/3C-Ap.Ser28SersryShT2m3c.13C-SAp.Leu/SMCmiAp2b2c.132G-Ap.Ag080HismiAfadyap11c.2696/7C-YmiTmray3c.2286/7Ty.MaBNAgySh152Mc.2286/7TmiPax5c.2286/7TmiPax6c.3261/3G-YmiActinc.1416-Ap.MaR/10miArbitc.1416-Ap.MaR/10miArbitc.1416-Ap.MaR/10miArbitc.1416-Ap.Pro31382rmiArbitc.1517-Cp.Pro31382rmiOffradc.5312,53-9delCAAAp.Pro31382rmiDiffsc.537-Cp.Pro31382rmiOffradc.537-Cp.Pro538anmiDiffsc.363-Ap.Afe28iemiOffradc.363-Cp.Dro31382rmiDiffsc.363-CTp.Dre308ianmiDiffsc.363-CTp.Dre308ianmiDiffsc.363-CTp.Dre308ianmiDiffsc.363-CTp.Dre308ianmiDiffsc.363-CTp.Dre308ianmiDiffsc.363-CTp.Dre308ianmiDiffsc.363-CTp.Dre308ianmiDiffsc.362-CTp.Dre308ianmiDiffsc.362-CTp.Dre308ianmiDiffsc.362-CTp.Dre308ian <td>intragenic_variant</td> <td>-</td> <td>0.438 (19/42)</td> <td>-</td> <td>-</td> <td>-</td> <td></td> <td>-</td> <td>-</td>	intragenic_variant	-	0.438 (19/42)	-	-	-		-	-
AppBbSC.14179G-AmmMyh9C.1437G-Cp.Leu479LeumyMyh9C.1437G-Cp.Leu479LeumyMyh9C.1437G-Ap.Leu479LeumyMyh2C.1523G-Ap.Lu49MetmiMarchanC.2586G-Tp.My68fsmiMarchanC.2786C-Tp.My88fsmyMyh1C.2286G-Tp.Matp12mySh3c21C.2286G-Tp.Matp12mySh3c21C.2286G-TmymyBhr2C.3305-Tp.Met71myRhr2C.345740u/Gp.Met71miGm2107n.1524du/Gp.Met71miGm440C.3312,S.3361CGTmimiGm440C.3312,S.3361CGTmimiGm440C.3312,S.3361CGTp.Pr081SermiGm1676G.263-Ap.Met71siaGm1676C.263-Ap.Met71siaGm1677C.263-Ap.Met71siaGm1678C.263-Ap.Met71siaGm163401.802-3898-DTmiGm163401.802-3898-DTmiGm163401.802-369-CTp.Pr08681.uGm163401.802-369-CTp.Pr03487.gGm163401.802-369-CTp.Pr03487.gGm163401.802-369-CTmiGm163401.802-369-CTmiGm163401.802-369-CTmiGm163401.802-369-CTmiGm163401.802-369-CTmiGm163401.802-369-CTmi <t< td=""><td>ntron_variant</td><td>-</td><td>0.411 (52/151)</td><td>-</td><td>-</td><td>-</td><td></td><td>-</td><td>-</td></t<>	ntron_variant	-	0.411 (52/151)	-	-	-		-	-
Myh9C.143°CACP.Leu/MetMyr9Slc17aBC.84G>AP.Sar2BSPrSyrSlc17aBC.13C>AP.Leu/MetminAlg2D2C.13C>AP.Jv68BfraAdryap110C.26967C>TImAdryap111C.26967C>TImThrap3C.2236C>TImSlc212C.2236C>TImSlc212C.2236C>TImSlc212C.2236C>TImAdryap11C.3695C>TImAdryap11C.3256C>TImActbC.141G>AP.Me147120C.141G>AP.Po131SerImActbC.141G>AP.Pr031SerImGrid4137n.15240GP.Pr031SerImGrid4137C.1521>CP.Pr031SerImGrid5766.305CTP.Pr031SerImGrid576C.1521>CP.Pr0408AImGrid576C.2586CTP.04969ValImDr1702C.2886CTP.04969ValImMyh202C.1038ACP.04969ValImMyh202C.1038ACP.04969ValImMyh202C.1038ACP.04969ValImAddC.144C>TP.14020MetImMyh202C.1038ACP.04963U,ImMyh202C.1038ACP.04963U,ImAddC.144C>TP.04963U,ImMyh202C.1038ACP.04963U,ImAddC.144C>TP.14270MetImAddC.144C>TP.14380ValImMyh	ntron_variant	-	0.408 (52/150)	-	-			-	
SintragiC. AlgoAD. SurgSermmTrav13-4v7c. 13CAD. LeuSMetmmMarba2c. 13C3AD. ArgoBellemmMarba2c. 1523G>AD. ArgoBellemmMarba2c. 268-67CmmmmTirrag3c. 2280-57J. Marba2sySind21c. 2280-57mmmmActbc. 2305-130-57J. Marba2mmActbc. 305-130-57mmmmActbc. 1183-2740-67P. Mot13NermmGm2117n. 115240-67P. Pot313SermmGm2147n. 115240-67P. Pot313SermmGm2147n. 115240-67P. Pot31SermmGm2147n. 115240-67P. Pot31SermmGm16576n. 880_681 delGTmmmmGm16576n. 2763-Ap. AlgoBellemmGm16576c. 2763-Ap. AlgoBellemmGm1657c. 2883-67p. AlgoBellemmGm1630n. 2690-57p. Dr 2080-67mmGm1630n. 2690-57p. Dr 2080-67mmGm1630n. 2690-57p. AlgoBellemmMarba2c. 2893-67p. AlgoBellemmMarba2c. 2893-67p. AlgoBellemmMarba2c. 2893-67p. AlgoBellemmMarba2c. 2893-67p. AlgoBellemmMarba2c. 2893-67p. AlgoBellemmMarba2c. 2893-67p. AlgoBellemmMarba2c. 2893-67	intron_variant synonymous_variant		0.093 (9/89) 0.082 (10/96)			-		- 0.075 (10/88)	
Tav13-dv07C.1523G>Ap.LuMetminAp2b2C.1523G>Ap.Arg508HisminAdvga11C.2666TC>TJ.Yr68isfraAdvga11C.2666TC>TJ.Arg503AppgyN32C11C.2278C>TgyPad3C.228C>TgyGm15524n.3629C>TgyGm15524n.3629C>TgyRihn2C.316S-TJ.Mat1C.111G>AD.Met77IIminGm24197n.1524dupGminGm4417n.1524dupGminGm4417C.1537-CP.Pr0313SerminGm4417C.1537-CP.Pr0313SerminGm10576G.800,614GIGTminGm10576G.263C>TP.Pr083AnminGm10576C.363C>TP.Pr083AnminGm10576C.363C>TP.C103ACminGm10576G.363C>TP.C103ACMinCapin2C.386C>TP.C1042PheminGm16340R.302-3808G>TminGm16340R.302-3806C>TP.C1042PheminMind2C.163ACP.C1042PheminMind4C.362C>TP.C1042PheminAddC.16449C>GJ.G1343U,minMind52C.395C>TP.G139LUminAdr34C.395C>TP.Mat20LUMinPich14C.283CAP.G139GVIMinPich24C.395C>TP.Mat20UAUMinPich24C.395C>TP.Mat20UAUMinPich24C.395C>TP.Ma	synonymous_variant		0.066 (9/146)	-	-	-		-	-
App2b2c.139.203upGATp.Agr09Hismm Marbmm Adacyap11c.2368-67-mm Marbmm Adacyap11c.2368-67-mm Marbmm Marbmm Marbmm Marbmm Marbmm Marbmm Marbmm Marbmm Marbmm Marbmm Marbmm 	missense_variant		0.058 (14/187)	-		-	-	-	
Memsc. 1.9.2.020.µGAATp. Tyrk88:fmAdayap 117c. 268-67C>TMak 22AspyrSh3c21c. 2278C>TP. Vall8ValyrSh3c21c. 2228C>TmyrPax9C. 2462AP. Vall8ValyrC. 305-13C>TmmfmAchc. 141G>AP.Mel47IemGm2137n. 1524dµGAP.F0313SermGm2147n. 1524dµGAP.F0313SermGr44c. 53-12, 53-9delCAAAP.F0513SermGr45c. 53-12, 53-9delCAAAp. Pre51SermGm10576n.860, 81 delGTmmGr45c. 275-Xp. Pre51SermGr45c. 248G>Ap. SerB3AnmGr45c. 248G>Ap. SerB3AnmGr47c. 248G>Ap. Pre58GmGr47c. 2693G>Tp. Pre58GmGr47c. 2693G>Tp. Pre58GmGr47c. 163AsCp. Pre58GmGr47c. 163AsCp. Pre58GmGr47c. 163AsCp. Pre53GmAdbc. 277G>Ap. Gu33GyAmAdbc. 177G>Ap. Gu33GyAmAdbc. 177G>Ap. Gu33GyAmAdbc. 171G>Ap. Gu33GyAmMark4c. 2233C>Tp. Gu33GyAmAdbfc. 113A-Gp. Gu37G'AmAdbfc. 113A-Gp. Gu37G'AmMark4c. 455C>Tp. Thr127Metm<	missense_variant		-	0.456 (38/83)					
Tring3C.228C>Tp.Ap932Aapp.ApSh3d21C.228C>Tp.Pax3C.228C>Tp.MattC.305-13C>Tm.AcbC.305-13C>Tm.AcbC.1163-47/upGm.MattC.1163-47/upGm.GrifAC.837-12, S3-94/07/AAm.GrifAC.837-12, S3-94/07/AAm.GrifAC.837-12, S3-94/07/AAm.GrifAC.837-12, S3-94/07/AAm.GrifAC.837-12, S3-94/07/AAm.GrifAC.837-12, S3-94/07/AAm.GrifAC.2840-Ap.Phe158rm.GrifAC.2840-CAp.Phe368-Lam.DrifAC.2840-CAp.Ser63Aanm.CaprinzC.2840-CAp.Pr0368-Lam.CaprinzC.2840-CAp.Pr0368-Lam.GrifA3A.820-STp.Fin208-Lam.GrifA3A.820-STp.Gu/982-Lam.Mybp2C.103A-Cp.Gu/982-Lam.GrifA3A.820-STp.Gu/982-Lam.Mybp2C.103A-Cp.Gu/982-Lam.ActhC.2230-STp.Gu/982-Lam.ActhC.2230-STp.Gu/982-Lam.Mybp2C.3950-STp.Gu/982-Lam.ActhC.3950-STp.Gu/982-Lam.ActhC.3950-STp.Gu/972-Lam.ActhC.3950-STp.Gu/972-Lam.ActhC.3950-STp.Gu/972-Lam.ActhC.3950-STp.Gu/972-	frameshift_variant&stop_gained	-	-	0.451 (46/109)	-	-	-	-	-
Si3621c.2462Ap.Val8ValsprPad9c.2462Ap.Val8ValsprGm15524n.3629C>TupRikn2c.3613G>TMat1c.14163Ap.Met7ImGm24107n.1524dupGupGm4417c.1184-70upGupGm4417c.1312,53-0alcCAAp.Pr0313SerminGridAc.5312,53-0alcCAAp.Pr0313SerminGridAc.537-Cp.Pr081SerminGridAc.537-Cp.Pr0851SerminGridAc.363-Ap.Pr0861SerminCapinAc.2680-STp.Pr0863AnminCapinAc.2680-STp.Clo9884AminCapinAc.2690-STp.Pr0863LauminCapinAc.1684-GAp.Clo9884AminMing1c.2690-STp.Pr0867LauminCapinAc.1684-GAp.Clo9884AminMing1c.1692-SBp.Pr0487DguMing1c.1692-SBp.Clo9851TminAddc.16449C>Gp.Clo9851NgminAddc.16449C>Gp.Glo391NgminAddc.113-SGp.Glo967GNguMinh2c.2892-STp.Glo967GNguMinh2c.2892-STp.Glo967GNguMinh2c.1013-SGp.Glo967GNguMinh2c.1013-SGp.Glo967GNguMinh2c.2892-STp.Glo967GNguMinh2c.2892-STp.Glo967GNguMinh2c.2892-STp.Glo967GN <td>intron_variant</td> <td></td> <td>-</td> <td>0.404 (27/63)</td> <td></td> <td>-</td> <td>-</td> <td>-</td> <td></td>	intron_variant		-	0.404 (27/63)		-	-	-	
ParsisC.246-Ap. Val8Valp.	synonymous_variant			0.349 (15/43)	-	-			
Gm15524n.3629C>TmmRkn2c.305-13G>TmmAcbC.141G>Ap.Met471emmGm2137n.1524dupGmmPr164C.1634/27dupGmmGr145C.53-12,53-9delCAAAp.Pr0313SermmGm16576n.680,8514GIGImmGm16576n.680,8514GIGImmGm16576c.235-12,53-9delCAAAp.Pr05158ermmGm16576n.680,8514GIGIp.Pr0526mmGm1576c.236Ap.Pr0526mmGm16360c.248G>Ap.Pr0586mmCapmin2c.288G>Tp.Pr0686LaummGm16340n.802-3898G>TmmGm16340c.16444G>Gp.Pr034Br0mmGm16340c.1644G>Gp.Pr034Br0mmAdhc.1614AG>Gp.Glu34JymmAdhc.2233C>Tp.Glu34JymmAdhc.2233C>Tp.Glu34G/GlymmAdh8c.113A>Gp.Aar38SermmAdh8c.113A>Gp.Glu34G/GlymmAdh8c.113A>Gp.Glu34G/GlymmAdh4c.382C>Tp.Glu34G/GlymmAdh4c.485C>Tp.Glu34G/GlymmAdh4c.485C>Tp.Glu34G/GlymmAdh4c.485C>Tp.Thr152MedmmAdh4c.485C>Tp.Thr152MedmmAdh54c.11424C>GmmmmAdh45c.1133-Gp.Clu36G/GlymmAdh46c.1132-Gp.Thr27Medmm <td>upstream_gene_variant synonymous_variant</td> <td>-</td> <td>-</td> <td>0.349 (15/43) 0.343 (19/50)</td> <td>-</td> <td>-</td> <td>-</td> <td>-</td> <td>-</td>	upstream_gene_variant synonymous_variant	-	-	0.349 (15/43) 0.343 (19/50)	-	-	-	-	-
Rin/2c.141G>Ap.Met47/lemitActbc.141G>Ap.Met47/lemitGm24197r.15240pGdoMast1c.152740pGP.Po313SerdoGridc.837C>TP.Po313SermitGridc.837C>Tp.Phe31SermitGridc.837C>Tp.Phe31SermitGridc.1527Cp.Phe51SermitGridc.3GsAp.Phe28lemitDiftc.3GsAp.Phe38lemitCaprinzc.2886C>Tp.Pho89k31mitGrif430c.162C>p.Pr0863AnmitGrif430c.162C>p.Pr0863AnmitGrif430c.162C>p.Pr0864AmitMybpc2c.1038ACp.Pr0364Pr0mitMybpc2c.1038ACp.Gu39UysmitAddc.144C>Gp.Gu39UysmitAddc.441G>Gp.Gu39UysmitAddc.441G>Gp.Gu39UysmitAddc.362C1CTp.Gu37USgatAnthc.332C4Cp.Ag134LumitAdthc.113A-Gp.Ag132LumitAnthéc.113A-Gp.Ag132LumitAnthéc.113A-Gp.Ag134LumitAnthéc.113A-Gp.Ag132LumitAnthéc.113A-Gp.Ag134LumitAnthéc.113A-Gp.Ag134LumitAnthéc.113A-Gp.Ag134LumitAnthéc.113A-Gp.Ag134S0mitPix0c.4	upstream_gene_variant			0.343 (19/50)		-			
ActbC141GAp.Melt47llemidGm24197n.11524dupGintPrpGc.1163+27dupGintPrpGc.53-12,534-04/CAAAintGm10576n.680_6814elGTintGm10576c.53-12,534-04/CAAAp.Phel5BerintGm10576c.78T5-Ap.Phel5BerintIlfismc.2680-GAp.Phel5BerintDrifac.2680-GAp.Phel2BilerintCaprinec.2480-GAp.Ser63AanintPhut111c.2693GSTp.Gby098ValintCaprinec.2680-GAp.Pro506BilerintOff1269c.23805-GTp.Fr068GaLuintGm16340n.802-3898G-GTp.Thr200MeRintGm16340n.802-3898G-GTp.Gu148C-GintMybpC2c.1038A-Cp.Pr0346Pr0styGraftc.16449C-GintintActbc.227G-Ap.Gu34JysintActbc.267G-Ap.Gu34JysintActbc.110G-Ap.Adg13LuintArk446c.455C-Tp.Gly36FilyintMytAc.321C-Tp.Gly36FilyintMytAc.321C-Tp.Gly36FilyintMytAc.321C-Tp.Gly36FilystyArk446c.455C-Tp.Thr27MetintMytAc.321C-Tp.Gly36FilyintMytAc.321C-Tp.Gly36FilyintMytAc.321C-Tp.Thr27MetintMytAc.321C-Tp.Thr27Met <td>ntron_variant</td> <td></td> <td></td> <td>0.333 (12/36)</td> <td></td> <td>-</td> <td>-</td> <td>-</td> <td>-</td>	ntron_variant			0.333 (12/36)		-	-	-	-
Masti 0.1184-27040G min PipG3 0.937C>T p.Pro313Ser min Grid 0.537L2, S3-061GAAA int find Grid/G7 0.1527L>C p.Phe51Ser min Olfr33 0.757LA p.Phe52Ber min Olfr33 0.757LA p.Phe52Ber min Dpt 2.248G-A p.Ser83Aan min Phof11 2.639G-T p.Dr098Ber min Caprin2 0.2886C>T p.Pr098Ber min Gmt6340 n.802-3896C>T p.Br098Ber min Mipbc2 1.196C-G p.Pr098Ber min Mipbc3 1.196C-G p.Oryd6Pr0 syr Alad 0.41440C-G min min Camin 0.236C-T p.Glu33Lys min Alad 0.41440C-G min min Camin 0.236C-T p.Glu33Lys min Mind 0.236C-T p.Glu33Lys min Phot6 0.1105-A p.	missense_variant			0.326 (18/56)		-			-
PipGis C.S3-12, S3-960/CAAA p.ProS13Ser min GridA C.S3-12, S3-960/CAAA min GridA C.S3-12, S3-960/CAAA min GridA C.S3-12, S3-960/CAAA p.PheS1Ser min GridAS C.S3-12, S3-960/CAAA p.PheS1Ser min GridAS C.275/A p.PheS1Ser min Diff C.2480-A p.SerS3An min Capin2 C.2880-ST p.ProS43Ser min Capin2 C.2880-ST p.ProS495Ma min GridAS c.3892-ST p.Lau42Phe min GridAS C.3083-SC p.ProS415N2 min GridAS c.3892-ST p.ProS415N2 min GridAS C.3912-SC p.ProS415N2 min Acda C.1614-SC min min Acda C.1614-SC min Mix Acda C.1614-SC min Mix Acda C.1614-SC p.Gio/3C/G Min Mada C.164-SC <td>downstream_gene_variant</td> <td>-</td> <td></td> <td>0.222 (10/44)</td> <td></td> <td>-</td> <td></td> <td>-</td> <td></td>	downstream_gene_variant	-		0.222 (10/44)		-		-	
GirldC.S.S.J. 2.S.S.GulCAAAmaxGm10576n.S.B0_6B1delGTAp.Phes158Gm10576c.S.S.C.S.C.S.C.S.C.S.C.S.C.S.C.S.C.S.C.	ntron_variant	-	-	0.222 (10/44)	-	-	-	-	
Gm10576n.880_081delGTp.Phe578midlghv8-8c.152T>Cp.Phe158rmidOlfr33c.78T>Ap.Phe128lermidDr1c.248G>Ap.Ser3AANmidPhd111c.288G>Tp.Ser3AANmidCaprin2c.288G>Tp.Pho68LaumidCaprin2c.288G>Tp.Pho68LaumidCaprin2c.288G>Tp.Pho68LaumidCaprin2c.288G>Tp.Pho68LaumidGm16340n.802-3898G>Tp.Pho36ProsyrSerg1c.192C>Gp.Pro36ProsyrSerg1c.192C>Gp.Pro36ProsyrSerg1c.16144G>GmidCamlc.410+DTp.Calin45'sstoMyh4c.3221C>Tp.Gin745'stoMyh4c.3221C>Tp.Gin745'stoMirkd46c.455C>Tp.Calin45'stoAlkd46c.455C>Tp.Gin745'stoMirkd4c.455C>Tp.Gin745'stoMirkd4c.455C>Tp.Gin745'stoMirkd4c.455C>Tp.Thr274MetmidGr13c.3434G>Tp.Gin745'stoSiz213c.1412+22C>GmidstoGr132Cc.3434G>Ap.Pro134rgmidZis132c.3434G>Ap.Pro134rgmidGr236Cp.SerG61SermidGr236Cr.2436C>Ap.Pro134rgmidGr336Cr.2436C>Ap.Pro134rgmidGr236Cr.2456C>Ap.Pro134rg <td>missense_variant ntron variant</td> <td>-</td> <td>-</td> <td>0.216 (22/106)</td> <td>-</td> <td>-</td> <td></td> <td>-</td> <td>-</td>	missense_variant ntron variant	-	-	0.216 (22/106)	-	-		-	-
ighviðð c.152°C p.Phe51Ser mm Difra3 c.7675A p.Phe28ile mm Difra3 c.7675A p.Phe28ile mm Difra c.365A p.Phe28ile mm Dift c.26805AT p.Gidy88Val mm Capin2 c.2886C>T p.Fod988Lau mm Gm16340 n.802-3889C>T p.Hot92DNet mm Olfr.720 c.1268AC p.Pr0348PrO sy Sarg1 c.1564AC p.Pr0348PrO sy Alad c.16449C>G p.Hot349Ly mm Actb c.2776SA p.Gu134Jy mm Actb c.2776SA p.Gu134Jy mm MyH4 c.3826>T p.Gu134Jy std MyH4 c.3826>T p.Gu134Gr std MyH4 c.3826>T p.Gu134Gr std MyH4 c.3826>T p.Gu134Gr std MyH4 c.3826>T p.Gu134Gr std MyH5 c.1342-G	non_coding_transcript_exon_variant			0.133 (8/63) 0.111 (12/94)					
Olfrido C.2675.A P.PR2011 Mattr 1115ra C.3GS-A p.Mattr siza Dpt C.3GS-A p.Mattr siza Dpt C.3GS-A p.Str63AA min PhotBill C.2888C>T p.Dr96861au min Capmic2 C.2888C>T p.Pr06961au min Str614A C.3888C>T min min Olfr1269 C.124C>T p.Lau42Phe min Mitpac2 C.138A>C p.Pr06361au min Alad C.4144C>G p.Glu33Lys min Alad C.4414G>G p.Glu33Lys min Alad C.4414G>G p.Glu33Lys min Alad C.4414G>G p.Glu33Lys min Alad C.4314G>G p.Glu33Lys min Alad C.4314G>G p.Glu33Lys min Alad C.4349C>T p.Th172Matt min Alad C.4349C>T p.Glu361GP y.Glu361GP Siza13 <tdc< td=""><td>missense_variant</td><td></td><td></td><td>0.059 (9/142)</td><td>-</td><td></td><td></td><td></td><td></td></tdc<>	missense_variant			0.059 (9/142)	-				
Dptc.2480GAp.Ser3AsnmmPihd111c.2690GATp.Fr0863LaummCaprin2c.2688GATp.Fr0868LaummNing1c.599CATp.Tr020MetmmClift2620c.124CATp.Hr0346ProseyMybpc2c.1038ACp.Pr0346ProseyStorp1c.195CAGp.Cy085TrgmmAladc.14149CAGp.Gy084ProseyCamlc.441GATp.Arg14LaummActbc.270GAAp.Gu343LyaummActbc.270GAAp.Gu343LyaummActbc.223GCATp.Gu343LyaummArbhc.362CATp.Lu1207LauseyMyh4c.362CATp.Arg14LauseyArbhc.113A-Gp.Arg38ErmmPiktoc.110GAAp.Diag37GlyseyArbh2c.439TAp.Diag37GlyseyArbh2c.133TACp.Diag37GlyseyPiktoc.103ACGp.Liag30ValseyPiktoc.133TACp.Diag361SiseyPiktoc.103ACAp.Nat880ValseySiz2a13c.141243CAGmmGar19c.824CATp.Nat80ValseyPixtoc.363CAGp.Pr0113ArgmmGra2c.333CAGp.Nat81AVAseyMittoc.362GAAp.Nat81AVAseyMittoc.363CAGp.Pr0113ArgmmTyc.363CAGp.Pr0113ArgmmMittoc.363CAGp.Nat61SAVAsey<	missense_variant		-	0.054 (9/178)	-		-		
Pind111 C.2893G>T p.04989Val mm Caprin2 C.2884C>T p.Pro368Lau mm Caprin2 C.2884C>T p.Pro368Lau mm Gm16340 n.802-3898G>T p.Pro368Pro mm Mitp22 C.1303A>C p.Pro346Pro syn Mitp22 C.1303A>C p.Pro346Pro syn Scrg1 C.16144C>G mi mi Cam C.41644C>G mi mi Carl C.277G>A p.Gu1674S* sto Myh4 C.3263C>T p.Gu1745* sto Myh4 C.3263C>T p.Gu1745* sto Atoh8 C.130A>G p.Ag132Lau mi Atoh4 C.450C>T p.Thr1570MC sto Pirk2 C.3995A>T p.Gly367Gly sto Atoh4 C.1101G>A p.Gly361Gly sty Parb C.1303T>G sto sto Cistari C.141242C>G mi sto Sto C.3994G>T	start_lost	-	-	-	0.381 (10/27)	-	-	-	-
Capin2 C.2890C>T P.Pro82Lau mmi Ntng1 C.599C>T P.Thr200Met mmi Capin23A0 n.802-3896C>T P.Thr200Met mmi Coll1230 C.126A>C P.Lu42Phe mmi Oll17200 C.136A>C P.Pro34FPO Syg Strg1 C.166HA P.Pro34FPO Syg Alad C.161A+G>C P.Arg164Leu mi Actb C.270SA P.Gu39Lya mi Actb C.270SA P.Gu19ZLeu mi Actb C.270SA P.Gu37Leu Syg Fam122C C.395G>T P.Arg13ZLeu mi Arbh C.113A>G P.Arg13ZLeu mi Mpdz C.495-CT P.Thr15ZMet mi Mrd2 C.495-CT P.Thr15ZMet mi Strg1 C.343-GC P.Gly66TGH Syg Pik3d C.1412-43C-G Int Syg Strg1 C.3973-27C-T T Mit Gar133 - C.27D-T P	missense_variant	-	-	-	0.294 (11/42)	-			-
Nng1 c.S90C-T p.Thr200Met mm Gm16340 n.802-39896-5 int Gm16340 c.124C-T p.Lau42Phe mm Mybpc2 c.1396A-C p.Pro34ePno sm Alad c.1461-49C-G int mm Caml c.491C-G p.Gu1942-0 mm Alad c.161-GA p.Gu1942-0 mm Atb c.2320-5T p.Gu1942-0 sp Myh4 c.3821C-T p.Gu1942-0 sp Myh4 c.3821C-T p.Gu1942-0 sp AthB c.1131-G p.Gu1947-0 p.Gu1947-0 sp Arkad6 c.4562-T p.Thr152Met mm Mpdz c.4997-A p.Gu1967G19 sp Mpdz c.3490-T sp1 mm Parb c.34390-T mm sp1 Mpdz c.34390-T mm sp1 Sp1 c.34390-T p.Gu1967G19 sp1 Sp1 c.34390-T p.Thr274Met	missense_variant	-	-	-	0.25 (26/103)	-	•	•	-
Cmft3d0n.802-3888G-TmitOlfr1280c.124C>Tp.Lau42PhemitMyboc2c.103A-Cp.Pr034BProsryScrg1c.103A-Cp.Pr034BProsryAddc.161449C>GmitCamlc.416447C>Gp.Glu34LysmitAddc.2271G>Ap.Glu34LysmitActbc.2271G>Ap.Glu34LysmitActbc.2230C>Tp.Glu34SPmitAthAc.3261C>Tp.Lau1207LeusryAthAc.3261C>Tp.Arg132LumitAthAc.313A-Gp.Arg132LumitAthAc.455C>Tp.Thr152MCmitMytAc.369G>Tp.Glu36TGNsryAthAc.455C>Tp.Thr152MCmitMytAc.3497C>TsrysryParbc.3497C>TsrysryParbc.3493C>Gp.Thr27MetmitSt2a13c.141242C>GmitsrySt2a14c.14242C>GmitsrySt2a15c.141242C>GmitmitGm23627c.8246>Tp.Thr27MetmitSt213c.141242C>Gp.Thr27MetmitSt2141c.1484C>Ap.Thr27MetmitSt2132c.141242C>Gp.Thr27MetmitSt2142c.141242C>Gp.Thr27MetmitSt2132c.141242C>Gp.Thr27MetmitSt2143c.141242C>Gp.Thr27MetmitSt2143c.141242C>Gp.Thr27Metmit <t< td=""><td>missense_variant missense_variant</td><td></td><td></td><td></td><td>0.241 (21/91) 0.238 (14/52)</td><td></td><td></td><td></td><td></td></t<>	missense_variant missense_variant				0.241 (21/91) 0.238 (14/52)				
Olfri 289 c.124C>T p.Lau2Phe mp Mybpc2 c.1038A>C p.Pro346Pro say Storg1 c.1038A>C p.Pro346Pro say Alad c.1444C>G p.Pro346Pro say Alad c.441G>T P.Arg144Lau mi Actb c.270SA p.Gu83Lya mi Actb c.2233C>T p.Gu745* sto Myh4 c.3621C>T p.Gu745* sto Atb8 c.113A>G p.Arg132Lau mi Myh4 c.3621C>T p.Arg132Lau mi Atb8 c.113A>G p.Arg142M mi Myd2 c.453C>T p.Arg142M mi Myd2 c.439T>A p.Gly66TiGI sy Silca11 c.1283A>G p.Gly66TiGI sy Silca13 c.14124.3C>G mi mi Gr19 c.8243A>G p.Gly86TiGI sy Silc312 c.141440.826SA mi mi Gr19 c.838C>G	intron_variant				0.235 (31/120)			-	
Singl C.195C>G p.Cys65Tpp mm Alad C.164449C>G mm Caml C.491G>T p.Gu53Uys mm Actb C.277G>A p.Gu53Uys mm Actb C.277G>A p.Gu53Uys mm Myh4 C.2233C>T p.Gu1745* dst Myh4 C.2233C>T p.Au732Lau sy Fam122C C.395G>T p.Au732Cau sy Anth46 C.113A-G p.Gu37G7G sy Anth46 C.456C>T p.Thr152MC mm Mpdz C.4987C>T p.Thr152MC mm Mpdz C.3949-C>T mm Gst2a13 c.141242C>G mm Sc2112 C.1373-C p.Ua130Val sy mm Sc2112 C.39742C>T mm Gst330Val sy Gr19 C.821C>T p.Va1830Val sy Gn73827C>T mm Gr19 C.821C>T p.Va1830Val sy Gn73827C>T mm Gr231A116	missense_variant				0.222 (35/161)	-			
Aladc.4444cSGminCamic.441GSTp.Ag164LagminCamic.247GSAp.Glu34JysminMathc.232GSTp.Glu34JysminUhrtc.232GSTp.Glu34JysminMyh4c.3621GSTp.Glu34JysminAladc.362GSTp.Glu34JysminMintAc.362GSTp.Ag132LauminPichc.1101GSAp.Glu367GiysynAnkrd6c.45GSTp.Thr152MetminPichc.3433TSCp.Glu367GiysynPickc.283ASGp.Glu361GiysynSiz2a13c.1412+32CSGp.Glu361GiysynSiz2a13c.1412+32CSGp.Thr27MetminGr19c.261CSTp.Thr27MetminFar2c.5334GSAp.Ser361SerminGm23GZc.7285GSAminsynTgc.3143-19GSGminTgc.3345-19GSAminTgc.3345-19GSAminTgc.3345-19GSAminTgc.3345-19GSAminTgc.3345-19GSAminTgc.3362Gp.Pro113ArgminSt13c.1984-126STAminSt13c.392-35minSt13c.192-35minSt13c.192-36CAminSt13c.192-36CAminSt13c.192-36CAminSt13c.192-36CAminSt13c.192-36CAminSt13c.192-36CA <td>synonymous_variant</td> <td></td> <td></td> <td>-</td> <td>0.213 (24/92)</td> <td></td> <td></td> <td>-</td> <td></td>	synonymous_variant			-	0.213 (24/92)			-	
Camin C.416-T p.4.0164Leu min Acb C.277G>A p.Glu3/31/s min Acb C.227G>A p.Glu3/31/s sta Myh4 C.322G>T p.Glu3/32/s sta Myh4 C.322G>T p.Lau1207Leu sty Atoh8 C.113A-G p.Ag132Leu min Atoh8 C.113A-G p.Ag132Leu min Atoh8 C.110A-G p.Gly367Giy sty Ankrd46 C.455C>T p.Thr152MC min Myh2 C.4997-A p.Bind67Lleu min Myh2 C.4997-A p.Bind67Lleu sty Parko C.3494-C>T sty sty sty Clain1 C.2883A-G p.Gly361Giy sty sty Sty121 C.3494-C>T p.Thr27Met min Gyr131 C.4282-CSC p.Thr27Met min Gyr1328 C.427C>T p.Thr27Met min Gyr1339 n.144491820G>A p.Gly361Ser min	missense_variant			-	0.192 (18/87)			-	
Actb C2770-A P.Gla93Lys mit Ubri C2233C>T p.Gln745'Lau spid Myh4 C32621C>T p.Gln745'Lau spid Fam12C C396C>T p.Agn7382-m mit Ath8 C.113A-G p.Agn7382-m mit Prich C.110-SA p.Agn7382-m mit Prich C.455C>T p.Thr152Met mit Mpd2 C.459C>T p.Byd67G) gr ParM0 C.499T>A p.Byd67G) gr C1811 C.2883A-G p.Byd67G) gr Glr19 C.2813A-G p.Gly661G) gr Glr19 C.812C>T p.Thr274Met mit Gr19 C.812C>T p.Va830Val gr Gm23627 C.1083C>T p.Ser361Ser gr Gm23627 C.1836C>A p.Por113Arg mit Tord C.3143-19O-C mit fm Gm24 C.3444-1982G>A p.Thr274Met mit Tord C.34	intron_variant	-	-	-	0.174 (27/139)	-	-	-	-
Uhri c.2320.5-T p.Gn/45* g.Gn Myh4 c.3621C>T p.Lou1207Leu syn Fam122c c.3621C>T p.Agri32Leu min Atoh8 c.130A-G p.Agri32Leu min Atoh8 c.130A-G p.Agri32Leu min Atoh8 c.130A-G p.Agri32Leu min Mpd2 c.495C>T p.Thr152Met min Mpd2 c.399T-A p.Phe17ile min Park0 c.39437C>T spi ga Vik2d c.31331A-G do do Ste213 c.1412+32C>G p.Thr27Met min Cycla c.3243C>T p.Thr27Met min Gra3627 n.2830S-A p.Ya830Val go Gra3627 n.2480G>A p.Ser361Ser go Zy11339 n.144491820S-A infi Gra2627 n.2480G>A p.Pr013Arg min Gra263 c.44845+1788C>T infi Gra264 c.44854+1788C>T infi </td <td>missense_variant</td> <td>-</td> <td></td> <td>-</td> <td>0.167 (8/48) 0.167 (10/64)</td> <td>-</td> <td>•</td> <td>-</td> <td></td>	missense_variant	-		-	0.167 (8/48) 0.167 (10/64)	-	•	-	
Myh4 c.385Cb-T p.Lat/207Lau symp Fam122c c.395Gb-T p.Arg132Lau mit Atoh8 c.113A-G p.Arg132Lau mit Prich c.113A-G p.Arg132Lau mit Prich c.113A-G p.Arg136G-G mit Prich c.455C-T p.Thr152MC mit Mpdz c.4457-C p.Phe167/1e mit Parb c.349-7C-T mit G St2cla13 c.1412-82C-G lint fritz Gyr19 c.821C>T p.Thr27Met mit St2h12 c.821C>T p.Val80Val gyr Gyr19 c.821C>T p.Val80Val gyr Gyr32627 n.283GA do gyr Gyr33627 n.2849C>T gyr gyr Gyr3427C>T p.Ser361Ser gyr gyr Gyr3427C>T p.Ser361Ser gyr gyr Gyr3427C>T p.Ser361Ser gyr gyr Gyr3436A n.348465	missense_variant stop_gained				0.154 (8/57)				
Fam122c c.389G>T p.Ag132Lau mit Atoha c.113A>G p.Ag138Ser mit Atoha c.1010G>A p.Okly667G/N syn Ankrd4 c.456C>T p.Thr152Met mit Mpdz c.489TSA p.Phe167lle mit Parb c.3949-7C>T sp1 sp1 Parb c.3949-7C>T sp1 sp1 Pikad c.3123T>C sp1 sp1 Sitz13 c.1412+32C>G mit sp1 Sp1 c.54349C>T p.Thr274Met mit Gp19 c.5421C>T p.Thr274Met mit Gp19 c.2490C>T y.VallS0Vall sp1 Zp133ps n.14449182G>A y.VallS0Vall sp1 Gm3313 n.14449182G>A mit sp1 Gm24 c.280-G p.Pr0113Arg mit Gm341 n.2480-SA p.Pr0113Arg mit Gm341 n.2480-SA p.Pr0113Arg mit Gm2 c.3124-GS	synonymous_variant				0.1 (8/47)				
Pickon c.10105A p.04/gerGly g.m Ankrd46 c.458C>T p.Thr152Met mit Mpdz c.458C>T p.Phe1671m mit Parbo c.458C>T p.Phe1671m mit Parbo c.2883A>G p.Phe1671m mit Parbo c.2883A>G p.04/g610g gy Pickod c.3133T>C p.04/g610g gy Sidza13 c.141243C>G mit mit Gpr19 c.821C>T p.Thr27Met mit Gpr19 c.821C>T p.Ma80Val gy Mdm32 c.973427C>T p.Ma80Val gy Mdm32 c.9836C>A p.Ser361Ser gy Mdm343 n.144441826D>A mit Gmr344 n.3484G>A up mit Yacc c.328C>G p.Pr0113Arg mit Gmr34 c.859.416S>A mit mit Gr13 c.465.465.276 gp mit S113 c.302C>T p.Mar78Arg	missense_variant				-	1.0 (56/56)			
Ankard46 c.455C>T p.Thr152Met min Mpdz c.4957C>T p.Pho171671/8 min Parbo c.9497C>T sp1 Cistri c.29397C>T sp1 Vikad c.31337C dot Sitz c.51337C dot Fu2 c.54349C>T min Fu2 c.54349C>T p.Thr27Met min Gyr19 6.821C>T p.Thr27Met min ZcSh12 c.97427C>T p.Val830Val spr Gyr392 n.72853G>A dot gor Zp1330 n.144491826C>A min gor Zp11342 p.346G>A p.Par01847 dot Zp11342 n.144491826C>A min gor Tg c.3143-169C infit gor min Gm214 n.144491826C>T infit gor min Sp1 c.521-302G3C>C infit gor min Sp1 c.521-302G3C>C infit gor gor <td>missense_variant</td> <td>-</td> <td></td> <td>-</td> <td>-</td> <td>0.676 (29/44)</td> <td></td> <td>-</td> <td></td>	missense_variant	-		-	-	0.676 (29/44)		-	
Mpdz c.4987-A p.Phe167/le mpl Parb c.949-7C>T sp0 Cish1 c.2883A-G p.Giy9617/le sp1 Cish1 c.2883A-G p.Giy9617/le sp1 Pik3c0 c.141242CsG int sp1 Sc2a13 c.141242CsG int mt Gpr19 c.821C>T p.Th/274Met int Sc2h12C c.973+27C>T p.Val830Val sp1 Gm23827 n.283GA Qm23827 dm Gm23827 n.184491829G>A dm gm34143 n.444491829G>A up Ync2 n.2384CsA p.Po113Arg mt gm4143 n.44491829G>A up Ync2 c.122-51G>A p.Po113Arg mt gm4144 n.44491829G>A up Ync2 c.123-63CA p.Po113Arg mt gm414 n.4465-46G> mt Ync2 c.1384-196C>T mt gm1 gm1 gm1 gm1 gm1 gm1 gm1 gm1 gm1 <td>synonymous_variant</td> <td>-</td> <td></td> <td>-</td> <td></td> <td>0.597 (46/77)</td> <td></td> <td>-</td> <td></td>	synonymous_variant	-		-		0.597 (46/77)		-	
Panb C4847C>T spip Clstn1 C2883A>G p.Gly61Gly spip Pikad C.3133T>C do do Sic2a12 C.141243C>G int do Sic2a13 C.141243C>G int do Sic2a13 C.141243C>G int do Sic2a12 C.54349C>T p.Th/274Met mit Gpr19 C.821C>T p.Yul830Val spi Z03t12 C.2490C>T p.Val830Val spi Z03t12 C.2493C>T Val830Val spi Z03t12 C.2493C>T p.SerJ61Ser spi Z015329 n.144491829C>A spi spi Gmax1 C.1083C>T p.Pro113drg mit Gmax1 C.521-03C> int spi Gr12 C.510-A int spi Gmax2 C.321-03C> p.Pro113drg mit Gmax1 C.485-3465-2ns spi spi S13 C.1982C>T p.MarAgrag	missense_variant	-		-	-	0.593 (17/32)	-	-	-
Cistmin c.283A>G p.GlydefGly grydefGly grydefGly <thgrydefgly< th=""> grydefGly <thgrydefgly< th=""> <thgrydefgly< th=""> <thgry< td=""><td>missense_variant splice_region_variant&intron_variant</td><td></td><td></td><td></td><td></td><td>0.534 (49/93) 0.517 (20/39)</td><td></td><td></td><td></td></thgry<></thgrydefgly<></thgrydefgly<></thgrydefgly<>	missense_variant splice_region_variant&intron_variant					0.534 (49/93) 0.517 (20/39)			
Pik3cd c.13137-C idd Si62a13 c.1412-32C-G int Fa2 c.543-9C-T int Gpr19 c.821-CT p.Th/27Met int Gpr14 c.973-92/CS-T int int Hydin c.9373-92/CS-T int int Gpr19 c.820-CT p.Val830/Val gor Gm23627 n.1283G-A Gor gor Dzahrl n.1484/05A up int McGa2 c.3285G-A up int Yacc2 n.2484/05A up int Strip n.3484/05A up int Yacc2 c.328-GA p.Pro113Arg int Gn2 c.338C-G p.Pro113Arg int Gn2 c.348-19G-C int int Ch23 c.448-1788C-AT int int Ch24 c.465-346-C gor gor int S13 c.330C-T p.Val11Val gor jor	synonymous_variant					0.5 (10/20)	0.368 (10/26)	0.6 (11/19)	0.6 (11/19)
Si2cla1 c.1412-a2C-G intt Fur2 c.54349C-T intt Gpr19 c.821C-T p.Thr274Met intt Zch12 c.97342/CsT Valla300/al sy Zch12 c.2490C5T p.Valla300/al sy Mark c.2490C5T p.Valla300/al sy Dzank1 c.2490C5T p.Ser361Ser sy Dzank1 c.1083C5T p.Ser361Ser sy Dzank1 n.144491829G5A intt Gmd3143 n.34445A up intt Gmd3143 n.34445A p.Pro113Arg intt Gmd314 c.3485-G p.Pro113Arg intt Gmd2 c.3143-1965-C intt intt Gmd31 c.3445-1980C>T intt intt Ch223 c.44854-17880C>T intt sp1 Ch323 c.44854-17880C>T sp1 sp1 Ch323 c.14872G-A p.Pro113Arg sp1 S13 c.1382C>G p.AntrAfArg sp	downstream_gene_variant		-		-	0.5 (10/20)	0.368 (10/26)	0.6 (11/19)	0.6 (11/19)
Gpr19 c.821C>T p.Thr274Met mt Zs3h12c c.973427C>T int Hydin c.2490C>T p.Val830Val syr Gm23827 n.2830G>A SerG31Ser syr Gm23827 n.2830G>A p.SerG31Ser syr Zjh132ps n.144491829G>A p.SerG31Ser syr Zjh133-ps n.144491829G>A int int Gm43143 n.3446G>A p.PerG13Arg int Gm4144 c.521-50SA int int Tg c.128-51G>A int int Gn42 c.521-50G>A int int Tg c.521-50G>A int int Tg c.521-50G>A int int Ch23 c.524-50G>C int int St13 c.1687-1686C>T int spl St13 c.1187-486C>A p.Thr478drg int Dp2a c.1167-48C>A p.Val11Val spl Dp2a c.1167-48C>A p.Val11Va	intron_variant		-	-		0.483 (14/31)			-
Za3hl2c 6.9742/Cs-T int int Hydin C.2490Cs-T y.Val830Val spy Gm23627 n.2853G-A do Dzank1 1.083Cs-T p.SerJ61Ser gy Zy11329c n.144491829Gs-A int Gm23627 n.144491829Gs-A int Gm2362 n.144491829Gs-A int Gm2362 c.12251Gs-A int Gm2362 c.12251Gs-A int Yacz c.124519Gs-C int Gmaz c.3143-19G-C int Gmaz c.3245-G p.Pr01134rg int Gmaz c.529-41G-A int int Ch23 c.4453-456-Sn spj p.131 c.192-Ga spj S13 c.1113_111-2ins spj p.132 c.1382-GA p.Mar/Mar/Mar spj Dip2a c.1167-34G-A p.Asr45Ara spj spj Dip2a c.1167-34G-A int Dip2a c.1167-34G-A int Dip2a	ntron_variant	-		-	-	0.477 (25/55)			-
Hydin C.2490C>T p.Val830Val syr Gm23627 n.72853G>A	missense_variant	-	-	-	-	0.463 (28/60)	-	-	-
Önzásé27 n.2883G-A Ónzásé27 n.2883G-A Ónzásé27 p.Sará61Ser Syr Dzank1 c.1083C>T p.Sará61Ser Syr Syr Dzahl n.144491829G-A Intr Syr Syr Ch23A13 n.144491829G-A Intr Syr Syr Syrc22 c.122-51G-A Intr Syr Syr Tg c.3143-196>C Intr Intr Gna2 c.3843-196>C p.Pro113Arg Intr Raph14 c.521-30023G>C Intr Intr Cd425 c.6465-3n Syr Syr S13 c.4465-465-2n Syr Syr S13 c.1380-CS p.Thr478drg Syr S13 c.1380-CS p.Shr43drg Syr S13 c.1433C>G p.Shr45Arg Syr Dip2a c.1167+34G>A Intr Syr Dip2a c.1167+34G>A Intr Syr Dip2a c.1167+34G>A Intr Syr	ntron_variant					0.429 (13/31)	- 0.404 (24/56)		
Dzanki c.1080-ST p.SerJ61Ser syr Zlp133-ps n.144491829GA inth Gm43143 n.3484GA up Xrcc2 c.2254GAA up Xrcc2 c.2254GAA up Strad c.3484J6GA up Xrcc2 c.2254GAA up Strad c.3484J6GAC inth Gnaz c.3484J6GAC nth Gnaz c.3283CA p.Pro113Arg mit Gnaz c.5394J6GAC inth f.0254GAC Kats c.4685-J485GAS spl p.114 c.1132GAC spl St13 c.4865-J485GAS spl p.114 spl p.11432GAC spl Zlp503 c.1432G-SG p.Thr478Arg spl p.11432GAC spl Zlp53 c.1187-3GS-AC p.Val11Val spl spl Dip2a c.1167-3GS-AC p.Mar64SAR spl Dip2a c.1167-3GS-AC inth Dip2a c.1167-3GS-AC i	synonymous_variant downstream_gene_variant					-	0.404 (24/56)		-
Zh133,ps n.4449/829G>A inth Gm43143 n3484G>A upp Sc22 c.122-51G>A inth Tg c.3143-19G>C inth Tg c.3143-19G>C inth Gm42 c.338C>G p.Po1134rg min Rsph14 c.521-30023G>C inth inth Vair c.599-41G>A p.Po1134rg min Ch232 c.4845-17886C>T inth f.167 S13 c.4855-17886C>T inth sp1 S13 c.1985-12859T>C inth sp1 S13 c.1132-111-2na sp1 sp1 S13 c.1132-517 p.Aanfs4Asn sp1 S14 c.11367-36C> p.Thr4780FQ inth S192 c.1167-36C>A inth sp1 S17 c.330C-1 p.Valt1Val sp1 S17 c.1382C>T p.Valt1Val sp1 S192 c.1167-36C>A inth S192 c.1167-36C>A inth <td>synonymous_variant</td> <td></td> <td></td> <td></td> <td></td> <td>-</td> <td>0.368 (10/27)</td> <td></td> <td>-</td>	synonymous_variant					-	0.368 (10/27)		-
Xro2 c.12.91G>A intt Tg c.3143.19G>C intt Gnaz c.338C>G p.Pro113Arg intt Rsph14 c.519.41G>C intt Stat c.509.41G>A intt Var c.509.41G>A intt Cdt23 c.4465-3465-21 intt St13 c.1959.41G>A spl St13 c.302.280-1ins spl Zh503 c.1343C>G p.Tri/78Arg spl Gabrb2 c.1382C>T p.Val11Va spl Dip2a c.1113.2111-2ns spl j Dip2a c.1167.43G>A mt j Dip2a c.1167.43G>A intt j Dip2a c.1167.43G>A intt j Ga050D2B1K c.7401148_1004-19delAG intt Ga050D2B1K c.780-T p.AlaS18Val intt Ga050D2B1K c.780-T p.AlaS18Val j Ga050D2B1K c.780-T p.AlaS18Val j Ga	ntragenic_variant	-	-	-	-	-	0.368 (10/27)	-	-
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	upstream_gene_variant	-		-	-	-	0.358 (26/68)	-	
Graz c.338-CG p.Pro113Arg mit Rsph14 c.521-30023G-C inth Rsph14 c.529-41G-A inth Cdh23 c.448-517886C-AT inth Cdh23 c.448-517886C-AT inth S13 c.4865-485-286 spl Pallc c.4865-485-286 spl S13 c.380-2_380-1ins spl S13 c.111-3_111-2ns spl Zhp503 c.1382G-ST p.Aar478Arg spl Olfr1375 c.330-G p.Thr478Arg spl Dip2a c.1167-43G-SA inth spl Dip2a c.1167-43G-SA inth spl Dip2a c.1167-43G-SA inth spl Dip2a c.1167-43G-SA inth spl Ga305D2D216 c.118243 inth spl Ga305D2D316 c.725AT p.Ala218Val inth Ga305D2D316 c.725AT p.Ala218Val spl Ga205D2D316 c.725AA do <td< td=""><td>ntron_variant</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>0.358 (26/68)</td><td>-</td><td>-</td></td<>	ntron_variant	-	-	-	-	-	0.358 (26/68)	-	-
Reph14 c.521:30023G>C intt Vsir c.509-41G>A intt Ch23 c.44945+1788C>T intt St13 c.44954-1788C>T intt St13 c.4953-4762Gars spi Palid c.1985+12850T>C intt St13 c.1167-311-2ns spi St13 c.1143C>G p.ValitVal spi Gabrb2 c.1382C>T p.ValitVal spi Olf137C c.33C>T p.ValitVal spi Dip2a c.1167-43G>A intt spi Dip2a c.1167-43G>A intt spi Dip2a c.1167-43G>A intt spi Sto11 c.1008+18_1008+19delAG intt intt GabrD20 c.1014+8_1401+19delAG intt intt GabrD20 c.1482T>A p.4a184a spi GabrD30223Rik c.78A>T p.4a26Ala spi GabrD40 c.2480T>A c.760Ser spi Gap200 c.5427T>A	ntron_variant missense variant			-			0.341 (37/105) 0.29 (10/36)		
Vsir c.509-41G>A inth Cdh23 c.4845+17896C>T inth St13 c.4865+17896C>T inth St13 c.4865-17896C>T inth St13 c.1865-17896C>T inth St13 c.1895-12859T>C inth St13 c.1890-2800-ins spl St13 c.1132, 111-2ins spl Gabro2 c.1433C>G p.Nth/78drg mith Dip2a c.1167-34G>A p.Val11Val spl Dip2a c.1167-43G>A inth inth Dip2a c.1167+34G>A inth inth Dip2a c.1167+34G>A inth inth Dip2a c.1167+34G>A inth inth Gar202 c.1167+34G>A inth inth Gm2702 c.1040+18_100+19delAG inth inth Gm2702 c.1040+18_100+19delAG inth inth Gm2702 c.1040+18_100+19delAG inth inth Gm2702 c.1040+18_100+19delAG	missense_variant ntron_variant			-	-	-	0.29 (10/36)	-	-
Cdh23 c.4845+17886C>T Intr St13 c.4853,465.2ns sp Pald c.1985+128951>C intr St13 c.1985,412891>C intr St13 c.1985,412891>C sp St13 c.1113,111-2ins sp Gabrb2 c.1433C>G p.Asrk5Asn sp Olfr375 c.330x7 p.Val11Val sp Dip2a c.1167+34G>A intr intr Dip2a c.1167+34G>A intr intr Gn2702 c.1167+34G>A intr intr Gn2703 c.1167+34G>A intr intr Gn2704 c.1167+34G>A intr intr Gn2705 c.1167+34G>A intr intr Gn2704 c.1167+34G>A intr intr Gn2705 c.1108+18_1008+19delAG intr intr Gn2705 c.1108+18_101+19delAG intr intr Gn2050023Rik c.78A>T p.Ala18Val syr Ergido	ntron_variant	-	-	-	-		0.286 (18/62)	-	
S113 c 465-3,465-2ns spl Palld c.1985+12859T>C intl S13 c.1985+12859T>C spl S13 c.1985-12859T>C spl S13 c.1167-311-2ns spl S143 c.11362-C p.Asn454Asn syl Gabrb2 c.1380-2,380-1ns ypl syl Gabrb2 c.1382-C p.Asn454Asn syl Dip2a c.1167-340-A intl p.Val11Val syl Dip2a c.1167-340-A intl f.1167-340-A intl Dip2a c.1167-340-A intl f.1167-340-A intl Gar202 c.1167-340-A intl f.1167-340-A intl Gn2702 c.1167-361-340-1540-1640-1640-1640-1640-1640-1640-1640-16	ntron_variant	-			-	-	0.286 (18/62)		
SH3 c.380-2.380-tins spl SH3 c.1143-SH2 spl SH3 c.1143-SH2 p.Arn45Asn mid Gabrb2 c.1382C>T p.Asn45Asn syp Olf17375 c.330-ST p.Val11Val syp Dip2a c.1167+34G>A inth bip2a c.1167+36C>A inth Dip2a c.1167+35T>A inth inth fmp Aarsd1 c.1008+18_1008+19delAG inth inth Sto11 c.1553C>T p.Ala518Val mith 6430550023Rik c.78A>T p.Ala548Val syp Ergic3 c.4283T>A c.4028T> dot Fry c.2820C>A p.Sar760Ser god Fry c.2820C>A p.Sar760Ser syp Gzme c.670SA p.Ala780Ser mith	splice_region_variant&intron_variant	-	-	-	-	-	0.214 (25/121)	-	-
St13 c.114.3_111-2ins spl Zip503 c.1433C>G p.Thr478drg mit Gabra2 c.1382C>T p.Asn45AaR syr Olf1375 c.3362C>T p.Val11Val syr Dip2a c.1167+34C>A inth Dip2a c.1167+34C>A inth Dip2a c.1167+34C>A inth Stord c.1167+34C>A inth Dip2a c.1167+34C>A inth Gm2702 c.1104+18,1004+19delAG inth Gm2702 c.1404+18,1004+19delAG inth Stort p.Ala18Val syr Stort c.4323T>A p.Ala26Aa syr Crigot c.4243T>A p.Ala26Aa syr Ergics c.4243T>A dor fry gr Fry c.2200c>A p.SerGoSer syr Grade p.SerGoSer gr gr Grup c.570c>A p.Ala780Ser mit	ntron_variant	-	-	-	-	-	0.203 (20/98)	-	-
Zip503 c.1433C>G p.Thr478Arg mit Gabrb2 c.1682C>T p.Asn45Asn syy Dip2a c.167+34G>A j.Val11Val syy Dip2a c.1167+34G>A inth Dip2a c.1167+36C>A inth Dip2a c.1167+36C>A inth Dip2a c.1167+36C>A inth Dip2a c.1167+35T>A inth Aarsd1 c.1008+18_1008+19delAG inth Gn27029 c.1401+18_141+19delAG inth G403650023Rik c.78A>T p.Ala518Val mit 6430560023Rik c.748AT p.Ala26Ala sy Ergic3 c.4283T>A od od Cp2500 c.*542TT>A do fry Gzme c.670>A p.Ser/60Ser sy Lin2b c.234C>G p.Asn78Us mit	splice_acceptor_variant&intron_variant				1	-	0.118 (11/105)		-
Gabric c1382C>T p_Asn45Asn sy Dlf1375 c.33C>T p_V4111Val syr Dlp2a c.1167-34G>A int Dlp2a c.1167-34G>A int Dlp2a c.1167-34G>A int Dlp2a c.1167-36T>A int Arad1 c.1008+18_1008+13delAG int Gm2702 c.1014+18_1401+13delAG int Sto11 c.1553C>T p_Ala518Val mit 6430550D23Flik c.78A>T p_Ala54As1 syr Ergic3 c.4283T>A ud do Cp2520 c.5428T>A do fry Gabros c.567O>A p_Ser/60Ser go Fry c.2820O>A p_Ser/60Ser mit Lre8b c.234C>G p_An78Lys mit	splice_region_variant&intron_variant missense_variant	-		-	-	-	0.07 (8/145)	- 0.545 (8/13)	-
Olfr1375 C33C>T p.Val11Val sy Dip2a c.1167+34G>A inth Sign c.1167+34G>A inth Gm27029 c.1004+18_1401+19delAG inth Gm27029 c.1401+18_1401+19delAG inth G430550D23Rik c.78A>T p.Ala25Nal sy Ergic3 c.4283T>A up dot Cp2520 c.5428T>A dot for Fry c.2280C>A p.Ser/GoSer sy Gzme c.670C>A p.Gly23SP mint Inrobb c.234C>G p.Asr/RUSSer mint	synonymous_variant				-			0.545 (8/13) 0.524 (23/45)	
Dip2a c.1167+34G>A inth Dip2a c.1167+34G>A inth Dip2a c.1167+34G>A inth Dip2a c.1167+36C>A inth Aarsd1 c.1081+36_1008+19delAG inth Gm27029 c.1401+19delAG inth Ston1 c.1553C>T p.Ala518Val min 6430550023Rik c.78A>T p.Ala58Val sy Ergic3 c.4283T>A up p.26250 c.*5427T>A do Fry c.2800>A p.Ser/60Ser grame c.67G>A p.Gly23Ser min Lröbb c.234C>G p.Asn7RUs min do do	synonymous_variant			-	-	-		0.44 (67/148)	-
Dip2a c.1167+35T>A inth Aarsd1 c.1008+18_1008+18408IAG inth Gm27029 c.1401+18_1401+19deIAG inth Ston1 c.1553C>T p.Ala518VaI min 6430550D28Rik c78A>T p.Ala518VaI min 6430550D28Rik c.78A>T p.Ala518VaI min 6430550D28Rik c.75427T>A p.Gar260Ser up Cap250 c.5427T>A do p.Ser760Ser syn Gizme c.670S-A p.Sel70Ser min Lröbb c.224C>G p.Asn78Lys min	intron_variant			-	-	-		0.127 (8/70)	
Aarsd1 c.1008+18_1008+19delAG inth Gm27029 c.1401+18_1401+19delAG inth Ston1 c.1553C5 p.Ala518Val mit 6430550023Rik c.78A>T p.Ala26Ala sys Ergic3 c.4283TA up c.92550 c.*5427T>A do Fry c.28005A p.Ser/60Ser sys g.grame c.670S-A p.Gly23Ser mit Lröbb c.224C-G p.Asn78Lys mit mit mit mit	intron_variant	-	-	-	-	-	-	0.127 (8/69)	-
Gm27029 c1401+18_1401+19delAG intil Stont c.1553C>T p.Ala518Val mix 630550D23Rik c.78A>T p.Ala26Ala sys Ergica c.4283T>A up p. Cop250 c.*5429T>A do do Fry c.2280S>A p.Ser/60Ser sys Gzma c.670GA p.Gl/92Ser mix Lrobb c.224C>G p.An78Lys mix	intron_variant		-	-		-	-	0.127 (8/68)	
Stort c.1553C>T p.Ala518Val mix 6430550023Rik c78A>T p.Ala26Ala syr pcglc3 c.4283T>A up Cep250 c.5427T>A do Fry c.28005>A p.Ser/60Ser syr Gzme c.6705>A p.Gly23Ser mix Lröbb c.224C>G p.Asn/78/ys mix	intron_variant					-		0.068 (11/146)	
6430550023Rik c.78A>T p.Ala26Ala syr Ergic3 c4283T>A up Cop250 c.*5427T>A do Fry c.28205>A p.Ser760Ser syr Gzme c.67G>A p.Gly23Ser mit Lre8b c.224C>G p.Asn78Uys mit	intron_variant	-						0.068 (11/146)	- 0.493 (37/79)
Ergic3 c4283T>A up Cep250 c.*5427T>A do Fry c.280G>A p.Ser760Ser sy Gzme c.676>A p.Gly23Ser mit Lrrc8b c.234C>G p.Asn78Lys mit	missense_variant synonymous_variant								0.493 (37/79) 0.457 (24/53)
Cep250 c.*5427T>A do Fry c.2280G>A p.Ser760Ser syn Gzme c.670S>A p.Gly23Ser mit Lrr8b c.234C>G p.Asn78Lys mit	upstream_gene_variant								0.457 (24/53)
Fry c.2280G>A p.Ser760Ser syr Gzme c.67G>A p.Gly23Ser mix Lrrc8b c.234C>G p.Asn78Lys mix	downstream_gene_variant		-	-		-	-	-	0.457 (24/53)
Gzme c.67G>A p.Gly23Ser mis Lrrc8b c.234C>G p.Asn78Lys mis	synonymous_variant			-	-	-		-	0.412 (8/20)
Lrrc8b c.234C>G p.Asn78Lys mit	missense_variant		-	-					0.396 (48/119)
Ccdc42 c.688C>T n Ara230Trn mis	missense_variant	-	-	-		-	-	-	0.378 (15/43)
	missense_variant	-	-	-	-	-	-	-	0.375 (16/39)
	downstream_gene_variant		-		-	-			0.125 (12/111)
	ntron_variant downstream_gene_variant								0.125 (12/111) 0.125 (12/108)
	ntron_variant	-		-		-	-	-	0.125 (12/108)

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Supplementary Table 2. Oligonucleotides

Genotyping primers

mKras_F4: mKras_R4: mKras R3: LSL-Kras (Y118-wt): LSL-Kras (Y117-LSL): LSL-Kras (Y116-common): Cre-F: Cre-R: ROSA A (common): ROSA B (transgene): ROSA C5 (wt): LSL-dTomato A (oIMR9020): LSL-dTomato B (oIMR9021): LSL-dTomato C (oIMR9103): LSL-dTomato D (oIMR9105): Trp53_F: Trp53_R:

GTTACCTCTATCGTAGGGTC ACACAAAGGTGAGTGTTAAAATATTGA AGTTTTTGATAATCTTGTGTGAGACA ATGTCTTTCCCCAGCACAGT CTAGCCACCATGGCTTGAGT TCCGAATTCAGTGACTACAGATG CCATCTGCCACCAGCCAG TCGCCATCTTCCAGCAGG AAAGTCGCTCTGAGTTGTTAT GCGAAGAGTTTGTCCTCAACC CCTCCAATTTTACACCTGTTC AAG GGA GCT GCA GTG GAG TA CCGAAAATCTGTGGGAAGTC GGCATTAAAGCAGCGTATCC CTGTTCCTGTACGGCATGG TTTTGAAGGCCCAAGTGAAG CCACTCACCGTGCACATAAC

Complementary oligonucleotides used for cloning sgRNAs

mKras-G12D-A:	CACCGTGGTTGGAGCTGATGGCGT
mKras-G12D-B:	AAACACGCCATCAGCTCCAACCAC
mKras-wt2-A:	CACCGAACTTGTGGTGGTTGGAGC
mKras-wt2-B:	AAACGCTCCAACCACCACAAGTTC
TP53-c-A:	CACCGACCCTGTCACCGAGACCCC
TP53-c-B:	AAACGGGGTCTCGGTGACAGGGTC

HDR ssDNA templates

G12R:

AGTTGTATTTTATTATTATTGTAAGGCCTGCTGAAAAATGACTGAGTATAAGCTTGTGGTGGTAGGAGCTCGAGGCGTAGGCAAGAGCGCCTTGACGATA-CAGCTAATTCAGAATCACTTTGTGGATGAGTATGACCC

G12C:

AGTTGTATTTTATTATTGTAAGGCCTGCTGAAAATGACTGAGTATAAGCTTGTGGTGGTGGAGCATGCGGTGTAGGCAAGAGCGCCTTGACGATA-CAGCTAATTCAGAATCACTTTGTGGATGAGTATGACCC

G12V:

AGTTGTATTTTATTATTATTGTAAGGCCTGCTGAAAAATGACTGAGTATAAGCTTGTGGTGGTGGCGCCGTAGGCGAGGCAAGAGCGCCTTGACGATA-CAGCTAATTCAGAATCACTTTGTGGATGAGTATGACCC

G12S:

AGTTGTATTTTATTATTATTGTAAGGCCTGCTGAAAAATGACTGAGTATAAGCTTGTGGTGGTGGCTGCGCGCTTCCGGAGTAGGCAAGAGCGCCTTGACGATA-CAGCTAATTCAGAATCACTTTGTGGATGAGTATGACCC

G12A:

AGTTGTATTTTATTATTATTGTAAGGCCTGCTGAAAATGACTGAGTATAAGCTTGTGGTGGTTGGCGCCGCGGGGCGTAGGCAAGAGCGCCTTGACGATA-CAGCTAATTCAGAATCACTTTGTGGATGAGTATGACCC

G13D:

AGTTGTATTTTATTATTATTGTAAGGCCTGCTGAAAATGACTGAGTATAAGCTTGTGGTGGTTGGAGCAGGAGACGTCGGCAAGAGCGCCTTGACGATA-CAGCTAATTCAGAATCACTTTGTGGATGAGTATGACCC

shRNA sequences

Nf1: TGCTGTTGACAGTGAGCGCAGGAATTATAATGCTTATCTATAGTGAAGCCACAGATGTATAGATAAGCATTATAATTCCTATGCCTACTGCCTCGGA

Ren.713: TGCTGTTGACAGTGAGCGCAGGAATTATAATGCTTATCTATAGTGAAGCCACAGATGTATAGATAAGCATTATAATTCCTATGCCTACTGCCTCGGA