Supplementary material

Gene Expression (mRNA) Markers for Differentiating between Malignant and Benign Follicular Thyroid Tumours

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Material—detailed description

Microarray study
Fresh-frozen (FF) material from 52 tumours (27 FTC, 25 FTA) was used for the microarray study. Surgical procedures on patients were performed in Polish and German centres at the MSC Memorial Cancer Center and Institute of Oncology in Gliwice (28 subjects), University of Leipzig, University of Halle, and Mainz University Hospital (24 subjects). The patient characteristics given below refer to the Gliwice group only. There were 26 women and 2 men in the Gliwice group, 23 diagnosed with FTA and 5 with FTC (among them 3 patients with widely invasive FTC). The mean age at diagnosis was 47.2 years, with a median of 45.5 years (range: 14–72 years). Eight subjects (among them 3 diagnosed with FTC) underwent primary total thyroidectomy, 15 underwent lobectomy, and the remaining 5 underwent thyroid lobectomy with partial or subtotal resection of a contralateral lobe. The mean and median tumour diameter were 27 mm and 23 mm, respectively (range: 10–55 mm). However, the tumour diameter was unknown in 3 patients. Two patients with FTA were also diagnosed with low risk papillary thyroid cancer. Complementary radiiodine (RAI) ablation was carried out in both patients who achieved complete remission. For the FTC patients, distant metastases (bones and lungs) were present at diagnosis in 2 patients, whereas one subject had local recurrence and distant metastases (mediastinum and lungs) 8 years after the initial diagnosis. Two were RAI-refractory, while RAI treatment with a cumulative activity of 500 mCi resulted in FTC stabilisation for another patient. One remaining FTC patient achieved complete remission after surgery and RAI ablation. The mean time to follow-up in the whole group was 3.7 years (range: 0–12 years).

Validation study
Forty patients were diagnosed with follicular thyroid adenoma (FTA subgroup) and 31 with follicular thyroid carcinoma (FTC subgroup). The FTA subgroup included 35 women and 5 men, with a mean age at diagnosis of 46.3 years and a median of 45 years (range: 19–79 years). Twenty-seven subjects underwent thyroid lobectomy, and 12 underwent total thyroidectomy. No information regarding the extent of surgery was identifiable for one patient. The median and mean tumour diameter were 27.5 mm and 30.2 mm, respectively (range: 10–80 mm). Twelve patients demonstrated no tumour recurrence, and one patient was diagnosed with nodular goitre in a contralateral thyroid lobe. For 27 remaining subjects there were no data related to the further course of the disease. The mean time to follow-up was 2 years (range 0–9 years).

The FTC subgroup included 21 women and 10 men, with a mean age at FTC diagnosis of 57 years, and a median of 59 years (range 24–83 years). Four patients were diagnosed with widely invasive FTC. The mean tumour diameter was 38.3 mm, with a median of 33.5 mm (range 15–110 mm). However, in 13 cases, no information regarding the tumour was provided. T1, T2, T3, and T4 features were diagnosed in 4, 11, 10, and 1 patient, respectively. Five remaining subjects were staged as Tx. Multifocal tumour growth was observed in 11 subjects. Four patients demonstrated lymph node metastases (N1) and 5 distant metastases at FTC onset. Twenty-four patients underwent total
thyroidectomy; among them primary total thyroid resection was carried out in 10 subjects and two stage procedure in 14 subjects. Six patients underwent surgery primarily due to nodular goitre a few years earlier (all of them finally had total thyroidectomy). Palliative surgery was performed in one remaining patient. All but one were treated with RAI. Seventeen subjects received therapeutic RAI activity only once, whereas 7 subjects required 2 courses of RAI ablation. Six patients with disseminated disease were treated with RAI 3 times or more. Seven patients were RAI refractory. Two subjects additionally underwent external beam radiation (one patient as a palliative procedure). One patient was given sorafenib. Finally, complete remission was observed in 18 patients, asymptomatic hyperthyroglobulinaemia in 3 subjects, partial regression in 1 subject, stable disease in 2 patients, and progressive disease in 6 patients. One patient died due to FTC. The mean time to follow-up was 7.4 years (range: 0–13 years).
**Figure S1.** Boxplots from the microarray data set (52 samples, both primary and secondary microarray datasets) with oncocytic and PDTC samples color-coded. Red: PDTC; blue: oncocytic. The solid circles represent samples with RAS mutation.
Figure S2. Boxplots from qPCR data (69 samples) with color-coded oncocytic features. Grey: non-oncocytic, blue: oncocytic. The solid circles represent samples with RAS mutation.
Figure S3. Principal component analysis of microarray data. First and second principal components are plotted. FTC samples are shown as triangles, and FTA samples are shown as circles. Samples with RAS mutation are shown in red; other samples are shown in blue.

Figure S4. Principal component analysis of microarray data. First and third principal components are plotted. FTC samples are shown as triangles, and FTA samples are shown as circles. Samples with RAS mutation are shown in red; other samples are shown in blue.
Figure S5. Histograms of qRT-PCR gene expression levels of all 15 genes. A. Normalized relative expression levels. B. Log-transformed normalized relative expression levels. These histograms enable a comparison of gene expression distribution before and after applying log-transformation.
**Table S1.** List of papers considered in the meta-analysis

| Reference       | Technology                        | Number of FTA samples | Number of FTC samples | Number of differentiating genes * |
|-----------------|-----------------------------------|-----------------------|-----------------------|----------------------------------|
| 1 Takano et al. 2000 [2] | SAGE                              | 1                     | 1                     | 4                                |
| 2 Barden et al. 2003 [3]    | Affymetrix microarray HG-U95      | 12                    | 7                     | 92                               |
| 3 Takano et al. 2004 [4]    | HDSS                              | 48                    | 29                    | 32                               |
| 4 Cerutti et al. 2004 [5]   | SAGE                              | 1                     | 1                     | 17                               |
| 5 Chevillard et al. 2004 [6]| cDNA microarrays                  | 4                     | 3                     | 43                               |
| 6 Weber et al. 2005 [7]     | Affymetrix microarray HG-U133A    | 12                    | 12                    | 77                               |
| 7 Taniguchi et al. 2005 [8] | ATAC-PCR                          | 45                    | 22                    | 53                               |
| 8 Lubitz et al. 2005 [9]    | Affymetrix microarray HG-U95Av2   | 14                    | 14                    | 13                               |
| 9 Fryknas et al. 2006 [10]  | cDNA microarrays                  | 10                    | 10                    | 22                               |
| 10 Stolf et al. 2006 [11]   | cDNA microarrays                  | 12                    | 12                    | 30                               |
| 11 Zhao et al. 2008 [12]    | cDNA microarrays                  | 12                    | 12                    | 130                              |
| 12 Hinsch et al. 2009 [13]  | Applied Biosystems Human Genome Survey Microarrays V 2.0 | 4                     | 8                     | 22                               |
| 13 Borup et al. 2010 [14]   | Affymetrix microarrays HG-U133 Plus 2.0 | 22                    | 18                    | 100                              |
| 14 Williams et al. 2011 [15]| Affymetrix HG-U133A               | 4                     | 15                    | 29                               |

Total 201 164

The number of samples describes the samples in the high-throughput gene expression experiments. The number of genes means the number of distinct genes for which it was possible to assign an Entrez ID.
Table S2. Detailed description of freshly frozen samples used for microarray experiment.

| SAMPLE  | SEX | AGE | ORIGIN | CLASS  | SET       | onco/PDTC | RAS   | DIAGNOSIS  |
|---------|-----|-----|--------|--------|-----------|-----------|-------|------------|
| FTC002  | F   | 74  | G      | FTC    | SECONDARY | NO        | NO    | primary    |
| FTC004  | F   | 80  | G      | FTC    | SECONDARY | NO        | NO    | primary    |
| FTC005  | M   | 66  | G      | FTC    | SECONDARY | NO        | NO    | primary    |
| FTC006  | F   | 78  | G      | FTA    | SECONDARY | NO        | NO    | 1 expert   |
| FTC010  | F   | 74  | G      | FTC    | SECONDARY | NO        | NO    | 2 experts  |
| FTC012  | M   | 50  | G      | FTC    | PRIMARY   | NO        | NO    | 2 experts  |
| FTC013  | F   | 61  | G      | FTC    | PRIMARY   | NO        | NO    | 2 experts  |
| FTC014  | F   | 72  | G      | FTC    | SECONDARY | NO        | NO    | 2 experts  |
| FTC015  | F   | 80  | G      | FTC    | SECONDARY | ONCO      | NO    | 2 experts  |
| FTC016  | F   | 61  | G      | FTC    | SECONDARY | NO        | NO    | primary    |
| FTC018  | M   | 38  | PL     | FTA    | SECONDARY | NO        | NO    | primary    |
| FTC020  | F   | 66  | PL     | FTC    | SECONDARY | NO        | NO    | primary    |
| FTC022  | F   | 47  | PL     | FTA    | SECONDARY | NO        | NO    | primary    |
| FTC024  | F   | 69  | PL     | FTC    | PRIMARY   | NO        | K61   | 2 experts  |
| FTC026  | F   | 44  | PL     | FTA    | SECONDARY | NO        | NO    | 2 experts  |
| FTC028  | F   | 72  | PL     | FTC    | PRIMARY   | ONCO      | NO    | 2 experts  |
| FTC030  | F   | 66  | PL     | FTC    | PRIMARY   | NO        | NO    | 2 experts  |
| FTC032  | F   | 34  | PL     | FTA    | PRIMARY   | NO        | NO    | 2 experts  |
| FTC034  | F   | 39  | PL     | FTC    | SECONDARY | NO        | unknown | 2 experts (lack of concordance) |
| FTC036  | F   | 23  | PL     | FTA    | SECONDARY | NO        | NO    | 2 experts  |
| FTC038  | F   | 14  | PL     | FTA    | PRIMARY   | NO        | NO    | 2 experts  |
| FTC040  | F   | 42  | PL     | FTA    | PRIMARY   | NO        | NO    | 2 experts  |
| FTC042  | F   | 29  | PL     | FTA    | PRIMARY   | NO        | NO    | 2 experts  |
| FTC044  | F   | 43  | PL     | FTA    | PRIMARY   | NO        | NO    | 2 experts  |
| FTC046  | F   | 31  | PL     | FTA    | PRIMARY   | NO        | NO    | 2 experts  |
| FTC048  | F   | 58  | PL     | FTA    | SECONDARY | NO        | NO    | 2 experts  |
| FTC050  | F   | 52  | PL     | FTA    | SECONDARY | NO        | NO    | 2 experts  |
| FTC052  | F   | 60  | PL     | FTA    | PRIMARY   | ONCO      | NO    | 2 experts  |
| FTC054  | F   | 69  | PL     | FTA    | PRIMARY   | ONCO      | NO    | 2 experts  |
| FTC058  | F   | 60  | PL     | FTA    | SECONDARY | NO        | NO    | 2 experts  |
| FTC060  | F   | 49  | PL     | FTA    | PRIMARY   | NO        | N61   | 2 experts  |
| FTC062  | M   | 67  | G      | FTC    | PRIMARY   | ONCO      | NO    | 2 experts  |
| FTC066  | M   | 68  | G      | FTC    | PRIMARY   | ONCO      | NO    | 2 experts  |
| FTC071  | M   | 72  | G      | FTC    | PRIMARY   | PDTC      | N61   | 2 experts  |
| FTC072  | F   | 66  | G      | FTC    | PRIMARY   | PDTC      | unknown | 2 experts  |
| FTC073  | M   | 61  | G      | FTC    | PRIMARY   | ONCO      | NO    | 2 experts  |
| FTC074  | -   | -   | G      | FTC    | SECONDARY | NO        | NO    | primary    |
| FTC075  | -   | -   | G      | FTC    | SECONDARY | NO        | NO    | primary    |
| FTC077  | M   | 76  | G      | FTC    | SECONDARY | PDTC      | N61   | 1 expert    |
| FTC078  | M   | 53  | G      | FTA    | SECONDARY | NO        | NO    | 2 experts  |
| FTC079  | F   | 61  | G      | FTC    | PRIMARY   | NO        | NO    | 2 experts  |
| FTC080  | M   | 61  | G      | FTC    | SECONDARY | PDTC      | NO    | 1 expert    |
| FTC081  | F   | 60  | G      | FTC    | PRIMARY   | ONCO      | NO    | 2 experts  |
| FTC082  | F   | 71  | G      | FTC    | PRIMARY   | ONCO      | NO    | 2 experts  |
| FTC083  | F   | 36  | G      | FTC    | SECONDARY | NO        | N61   | 1 expert    |
| FTC085  | F   | 71  | PL     | FTA    | PRIMARY   | NO        | NO    | 2 experts  |
| FTC086  | F   | 55  | PL     | FTA    | PRIMARY   | NO        | NO    | 2 experts  |
| FTC376  | F   | 71  | PL     | FTA    | SECONDARY | NO        | NO    | 2 experts  |
| FTC385  | M   | 33  | PL     | FTA    | SECONDARY | NO        | NO    | 2 experts  |
| FTC390  | F   | 33  | PL     | FTA    | PRIMARY   | NO        | NO    | 2 experts  |
| FTC392  | F   | 25  | PL     | FTA    | SECONDARY | NO        | NO    | 2 experts  |
| SAMPLE   | SEX | AGE | CLASS | RAS | ONCO |
|----------|-----|-----|-------|-----|------|
| PFA1     | F   | 54  | FTA   | N61 | 0    |
| PFA10    | F   | 62  | FTA   | 0   | 1    |
| PFA104   | F   | 42  | FTA   | 0   | 0    |
| PFA105   | F   | 23  | FTA   | 0   | 1    |
| PFA106   | F   | 24  | FTA   | 0   | 0    |
| PFA11    | F   | 44  | FTA   | 0   | 1    |
| PFA110   | F   | 74  | FTA   | 0   | 0    |
| PFA112   | M   | 45  | FTA   | 0   | 0    |
| PFA113   | F   | 51  | FTA   | 0   | 0    |
| PFA117   | F   | 74  | FTA   | 0   | 0    |
| PFA118   | F   | 44  | FTA   | 0   | 0    |
| PFA120   | F   | 58  | FTA   | 0   | 0    |
| PFA122   | F   | 25  | FTA   | unknown | 0 |
| PFA13    | F   | 52  | FTA   | unknown | 0 |
| PFA17    | F   | -   | FTA   | 0   | 0    |
| PFA19    | F   | 27  | FTA   | 0   | 0    |
| PFA23    | M   | 57  | FTA   | 0   | 0    |
| PFA28    | F   | 40  | FTA   | 0   | 0    |
| PFA3     | F   | 30  | FTA   | 0   | 0    |
| PFA37    | F   | 42  | FTA   | unknown | 0 |
| PFA38    | F   | 19  | FTA   | 0   | 0    |
| PFA4     | F   | 79  | FTA   | K12 | 1    |
| PFA46    | F   | 68  | FTA   | unknown | 0 |
| PFA54    | F   | 37  | FTA   | N61 | 1    |
| PFA6     | F   | 36  | FTA   | H61 | 0    |
| PFA62    | F   | 63  | FTA   | 0   | 0    |
| PFA65    | F   | 21  | FTA   | 0   | 0    |
| PFA66    | F   | 41  | FTA   | unknown | 0 |
| PFA68    | F   | 52  | FTA   | unknown | 0 |
| PFA73    | M   | 32  | FTA   | 0   | 0    |
| PFA75    | F   | 48  | FTA   | 0   | 0    |
| PFA8     | F   | 23  | FTA   | 0   | 0    |
| PFA80    | M   | 32  | FTA   | unknown | 0 |
| PFA92    | F   | 43  | FTA   | 0   | 0    |
| PFA95    | F   | 66  | FTA   | 0   | 0    |
| PFA96    | F   | 45  | FTA   | 0   | 0    |
| PFTC13   | F   | 30  | FTC   | 0   | 0    |
| PFTC14   | F   | 54  | FTC   | N61 | 0    |
| PFTC18   | F   | 58  | FTC   | 0   | 1    |
| PFTC19   | M   | 52  | FTC   | H12 | 0    |
| PFTC20   | F   | 54  | FTC   | 0   | 1    |
| PFTC24   | F   | 59  | FTC   | 0   | 0    |
| PFTC31   | F   | 65  | FTC   | 0   | 1    |
| PFTC33   | M   | 44  | FTC   | 0   | 1    |
| PFTC34   | F   | 53  | FTC   | 0   | 1    |
| PFTC38   | F   | 49  | FTC   | 0   | 1    |
| PFTC39   | F   | 75  | FTC   | N61 | 0    |

Table S3. Detailed description of 71 FFPE samples used for qRT-PCR validation experiment.
Table S4. Significantly differentially expressed genes based on analysis of our own microarray dataset. Genes marked in grey were selected for QPCR validation.

| Gene Id       | Symbol       | Name                                                                 | p-value in primary data set | Fold change in primary data set | p-value in secondary data set | Fold change in secondary data set |
|---------------|--------------|----------------------------------------------------------------------|-----------------------------|---------------------------------|-----------------------------|---------------------------------|
| 219929_s_at  | ZFYVE21      | zinc finger, FYVE domain containing 21                              | 8.68E-06                    | 0.41                            | 1.90E-03                    | 0.40                            |
| 201781_s_at  | AIP          | aryl hydrocarbon receptor interacting protein                      | 1.29E-05                    | 0.55                            | 7.70E-03                    | 0.63                            |
| 224445_s_at  | ZFYVE21      | zinc finger, FYVE domain containing 21                              | 2.32E-05                    | 0.50                            | 9.80E-03                    | 0.62                            |
| 217475_s_at  | LIMK2        | LIM domain kinase 2                                                | 2.55E-05                    | 0.35                            | 3.26E-03                    | 0.54                            |
| 1553313_s_at | SLC5A3       | solute carrier family 5 (sodium/myo-inositol cotransporter), member 3 | 3.32E-05                    | 0.26                            | 8.91E-04                    | 0.35                            |
| 210582_s_at  | LIMK2        | LIM domain kinase 2                                                | 3.59E-05                    | 0.36                            | 8.03E-04                    | 0.58                            |
| 226950_at    | ACVRL1       | activin A receptor type II-like 1                                   | 4.94E-05                    | 0.35                            | 4.82E-03                    | 0.49                            |
| 200648_s_at  | GLUL         | glutamate-ammonia ligase                                           | 5.05E-05                    | 0.33                            | 3.11E-02                    | 0.48                            |
| 218723_s_at  | C13orf15     | chromosome 13 open reading frame 15                                | 5.06E-05                    | 0.28                            | 3.27E-03                    | 0.45                            |
| 217543_s_at  | MBTPS1       | membrane-bound transcription factor peptidase, site 1              | 5.81E-05                    | 0.49                            | 4.71E-04                    | 0.58                            |
| 209046_s_at  | GABARAPL2    | GABA(A) receptor-associated protein-like 2                         | 8.30E-05                    | 0.58                            | 6.35E-04                    | 0.68                            |
| 217202_s_at  | GLUL         | glutamate-ammonia ligase                                           | 9.49E-05                    | 0.34                            | 1.25E-02                    | 0.47                            |
| 222670_s_at  | MAFB         | v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)    | 9.56E-05                    | 0.34                            | 1.03E-02                    | 0.56                            |
| 205200_at    | CLEC3B       | C-type lectin domain family 3, member B                             | 1.04E-04                    | 0.25                            | 1.02E-02                    | 0.35                            |
|   |   |   | ATG13 autophagy related 13 homolog (S. cerevisiae) |   |   |   |
|---|---|---|-----------------------------------------------|---|---|---|
|15 | 203364_s_at | ATG13 | 1.15E-04 | 0.52 | 2.50E-02 | 0.70 |
|16 | 214040_s_at | GSN | gelsolin | 1.60E-04 | 0.22 | 4.09E-03 | 0.26 |
|17 | 213164_at | NA | NA | 1.64E-04 | 0.43 | 6.78E-03 | 0.49 |
|18 | 215749_s_at | GORASP1 | golgi reassembly stacking protein 1, 65kDa | 1.74E-04 | 0.58 | 3.56E-02 | 0.67 |
|19 | 203240_at | FCGBP | Fc fragment of IgG binding protein | 1.77E-04 | 0.20 | 3.34E-02 | 0.30 |
|20 | 201782_s_at | AIP | aryl hydrocarbon receptor interacting protein | 1.90E-04 | 0.64 | 2.52E-02 | 0.80 |
|21 | 229074_at | EHD4 | EH-domain containing 4 | 1.94E-04 | 0.36 | 3.26E-02 | 0.60 |
|22 | 211672_s_at | ARPC4 | actin related protein 2/3 complex, subunit 4, 20kDa | 2.08E-04 | 0.46 | 1.61E-02 | 0.57 |
|23 | 206113_s_at | RAB5A | RAB5A, member RAS oncogene family | 2.22E-04 | 0.53 | 3.96E-02 | 0.61 |
|24 | 219238_at | PIGV | phosphatidylinositol glycan anchor biosynthesis, class V | 2.28E-04 | 0.50 | 3.56E-03 | 0.61 |
|25 | 209021_x_at | ATG13 | ATG13 autophagy related 13 homolog (S. cerevisiae) | 2.30E-04 | 0.63 | 8.43E-03 | 0.67 |
|26 | 1554159_a_at | ZMYND11 | zinc finger, MYND-type containing 11 | 2.65E-04 | 0.37 | 9.49E-04 | 0.42 |
|27 | 221619_s_at | MITCH1 | mitochondrial carrier 1 | 2.83E-04 | 0.50 | 1.24E-02 | 0.58 |
|28 | 204246_s_at | DCTN3 | dynactin 3 (p22) | 3.17E-04 | 0.61 | 1.46E-03 | 0.62 |
|29 | 212944_at | SLC5A3 | solute carrier family 5 (sodium/myo-inositol cotransporter), member 3 | 3.20E-04 | 0.38 | 3.71E-03 | 0.43 |
|30 | 220589_s_at | ITFG2 | integrin alpha FG-GAP repeat containing 2 | 3.21E-04 | 0.62 | 9.15E-03 | 0.56 |
|31 | 216004_s_at | PKNOX1 | PBX/knotted 1 homeobox 1 | 3.48E-04 | 0.43 | 1.17E-02 | 0.70 |
|32 | 241874_at | C5orf53 | chromosome 5 open reading frame 53 | 3.79E-04 | 0.44 | 1.76E-02 | 0.54 |
|33 | 213167_s_at | SLC5A3 | solute carrier family 5 (sodium/myo-inositol cotransporter), member 3 | 3.86E-04 | 0.20 | 1.24E-03 | 0.23 |
|34 | 215357_s_at | POLDIP3 | polymerase (DNA-directed), delta interacting protein 3 | 4.19E-04 | 0.41 | 1.05E-02 | 0.54 |
|35 | 224732_at | CHTF8 | CTF8, chromosome transmission fidelity factor 8 homolog (S. cerevisiae) | 4.24E-04 | 0.57 | 2.02E-02 | 0.72 |
|36 | 1553243_at | ITIH5 | inter-alpha (globulin) inhibitor H5 | 4.55E-04 | 0.25 | 8.88E-03 | 0.40 |
|37 | 204049_s_at | PHACTR2 | phosphatase and actin regulator 2 | 4.63E-04 | 0.46 | 2.93E-02 | 0.63 |
|38 | 211383_s_at | WDR37 | WD repeat domain 37 | 4.65E-04 | 0.55 | 1.45E-02 | 0.78 |
|39 | 209652_s_at | PGF | placental growth factor | 4.68E-04 | 0.20 | 2.69E-02 | 0.35 |
|40 | 224872_at | DIP2B | DIP2 disco-interacting protein 2 homolog B (Drosophila) | 4.89E-04 | 1.78 | 1.98E-02 | 1.33 |
|41 | 222507_s_at | TMEM9B | TMEM9 domain family, member B | 5.20E-04 | 0.55 | 2.36E-03 | 0.54 |
|42 | 212890_at | SLC38A10 | solute carrier family 38, member 10 | 5.35E-04 | 0.53 | 1.99E-02 | 0.67 |
|43 | 233140_s_at | VIPAR | VPS33B interacting protein, apical-basolateral polarity regulator | 5.36E-04 | 0.61 | 7.34E-03 | 0.82 |
|44 | 1554451_s_at | DNAJC14 | DnaJ (Hsp40) homolog, subfamily C, member 14 | 5.57E-04 | 0.41 | 2.29E-03 | 0.49 |
|   |   |   |   |   |
|---|---|---|---|---|
| 45 | 1555434_at | SLC39A14 | solute carrier family 39 (zinc transporter), member 14 | 5.69E-04 | 0.35 | 8.90E-03 | 0.36 |
| 46 | 210622_x_at | CDK10 | cyclin-dependent kinase 10 | 5.98E-04 | 0.27 | 1.78E-02 | 0.53 |
| 47 | 213331_at | NA | NA | 6.12E-04 | 0.38 | 1.55E-02 | 0.49 |
| 48 | 212914_at | CBX7 | chromobox homolog 7 | 6.38E-04 | 0.35 | 1.02E-02 | 0.48 |
| 49 | 242137_at | RBMS3 | RNA binding motif, single stranded interacting protein 3 | 6.38E-04 | 0.23 | 1.95E-02 | 0.36 |
| 50 | 203188_at | B3GNT1 | UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1 | 6.55E-04 | 0.50 | 4.65E-02 | 0.72 |
| 51 | 213788_s_at | NA | NA | 6.93E-04 | 0.51 | 3.97E-02 | 0.70 |
| 52 | 201620_at | MBTPS1 | membrane-bound transcription factor peptidase, site 1 | 6.98E-04 | 0.51 | 3.89E-03 | 0.58 |
| 53 | 222510_s_at | MKRN2 | makorin ring finger protein 2 | 7.13E-04 | 0.42 | 1.38E-03 | 0.53 |
| 54 | 217427_s_at | HIRA | HIR histone cell cycle regulation defective homolog A (S. cerevisiae) | 7.19E-04 | 0.26 | 5.59E-03 | 0.44 |
| 55 | 204800_s_at | DHRS12 | dehydrogenase/reductase (SDR family) member 12 | 7.20E-04 | 0.46 | 2.62E-04 | 0.54 |
| 56 | 203222_s_at | TLE1 | transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila) | 7.79E-04 | 0.33 | 1.58E-02 | 0.46 |
| 57 | 210734_x_at | MAX | MYC associated factor X | 7.80E-04 | 0.54 | 3.16E-02 | 0.60 |
| 58 | 202278_s_at | SPTLC1 | serine palmitoyltransferase, long chain base subunit 1 | 7.97E-04 | 0.45 | 8.78E-04 | 0.49 |
| 59 | 220633_s_at | HP1BP3 | heterochromatin protein 1, binding protein 3 | 8.07E-04 | 0.64 | 1.11E-02 | 0.68 |
| 60 | 210524_x_at | NA | NA | 8.08E-04 | 0.28 | 2.81E-02 | 0.28 |
| 61 | 200894_s_at | FKB4 | FK506 binding protein 4, 59kDa | 8.09E-04 | 0.50 | 2.20E-02 | 0.62 |
| 62 | 204368_at | SLCO2A1 | solute carrier organic anion transporter family, member 2A1 | 8.27E-04 | 0.37 | 2.09E-02 | 0.51 |
| 63 | 233528_s_at | NA | NA | 8.89E-04 | 0.57 | 1.94E-03 | 0.55 |
| 64 | 217165_x_at | MT1F | metallothionein 1F | 8.94E-04 | 0.22 | 9.77E-03 | 0.22 |
| 65 | 238561_s_at | UTP23 | UTP23, small subunit (SSU) processome component, homolog (yeast) | 8.98E-04 | 0.43 | 1.72E-02 | 0.60 |
| 66 | 217853_at | TNS3 | tensin 3 | 9.03E-04 | 0.43 | 4.84E-03 | 0.50 |
| 67 | 210144_at | TBC1D22A | TBC1 domain family, member 22A | 9.38E-04 | 0.55 | 3.03E-02 | 0.67 |
| 68 | 227615_at | NA | NA | 9.49E-04 | 0.40 | 4.59E-03 | 0.49 |
| 69 | 204186_s_at | PPID | peptidylprolyl isomerase D | 9.60E-04 | 0.53 | 3.32E-03 | 0.56 |
| 70 | 213629_x_at | MT1F | metallothionein 1F | 9.63E-04 | 0.23 | 2.22E-02 | 0.23 |
| 71 | 218559_s_at | MAFB | v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian) | 9.68E-04 | 0.37 | 1.76E-03 | 0.50 |
| 72 | 203522_at | CCS | copper chaperone for superoxide dismutase | 9.76E-04 | 0.51 | 9.55E-03 | 0.63 |
Table S5. Primer design for RAS mutation profiling. Three first primers marked “_FF” were used for freshly frozen materials and all regions containing 3 mutation sites were amplified. Other primers were used for the FFPE materials.

| Gene/primer       | Forward primer                  | Reverse primer                  | Application | Mutation codons |
|-------------------|---------------------------------|---------------------------------|-------------|-----------------|
| HRAS_FF           | 5′-CAGGAGACCCTGTAGGAGGA-3′       | 5′-CTTCACCCCGTTTGATCTGCT-3′     | cDNA        | HRAS 12,13,61   |
| KRAS_FF           | 5′-GGAGAGAGGCCCTGCTGAA-3′       | 5′-AAAGAAAGCCCTCCGACGT-3′      | cDNA        | KRAS 12,13,61   |
| NRAS_FF           | 5′-CTGTCAAAAGCAGAGGAGC-3′       | 5′-TGGCAGATACACAGAGGAAGC-3′    | cDNA        | NRAS 12,13,61   |
| HRAS_1213_1       | 5′-TGAGGAGCGATGACGGAATA-3′      | 5′-GGGTCTATTGCTCCACAA-3′       | DNA, cDNA   | HRAS 12,13      |
| HRAS_1213_2       | 5′-CAGGAGACCCTGTAGGAGGA-3′      | 5′-ATCAATGACCCACCTCGTCC-3′     | cDNA        | HRAS 12,13      |
| Kras_1213         | 5′-AGCCCTGCTGAAAATGACTG-3′      | 5′-TTGGATCATATTCGCTCCACAA-3′   | DNA         | KRAS 12,13      |
| NRAS_1213_1       | 5′-GCAGTGGAGCGTGGAGTTCT-3′      | 5′-AAGTGGATTCTGGATTAGCTGGA-3′ | cDNA        | NRAS 12,13      |
| NRAS_1213_2       | 5′-TTACTGGTTCCACACGAGGTTCT-3′  | 5′-TGGAATTGTCACTGCGCTTT-3′     | DNA         | NRAS 12,13      |
| HRAS_61           | 5′-GTGTCATTGATTGGGAGGAC-3′      | 5′-GCAAACACACACAGGAAGC-3′      | DNA, cDNA   | HRAS 61         |
| Kras_61           | 5′-TGTTGTTTCTCCCTCTTCCAGGA-3′  | 5′-TGGCAATACACAAAGAAGAC-3′     | DNA         | Kras 61         |
| NRAS_61           | 5′-CAAGTGGTTATAGATGGTGAACC-3′  | 5′-TCGCGCTGCTCTCATGTATTG-3′    | DNA, cDNA   | NRAS 61         |

Table S6. Genes selected for qRT-PCR validation

| Gene symbol | Gene name                               |
|-------------|-----------------------------------------|
| PLVAP       | plasmlemma vesicle associated protein    |
| CPQ (PGCP)  | carboxypeptidase Q                       |
| ACVR1L1     | activin A receptor type II-like 1        |
| CLEC3B      | C-type lectin domain family 3, member B  |
| DIP2B       | DIP2 disco-interacting protein 2 homolog B |
| GABARAP1L2  | GABA(A) receptor-associated protein-like 2 |
| LIMK2       | LIM domain kinase 2                      |
| ZFYVE21     | zinc finger, FYVE domain containing 21   |
| ZMYND11     | zinc finger, MYND-type containing 11     |
| MAFB        | v-maf musculoaponeurotic fibrosarcoma oncogene homolog B |
| EGR2        | early growth response 2                  |
| FAM189A2    | family with sequence similarity 189, member A2 |
| SLC26A4     | solute carrier family 26 (anion exchanger), member 4 |
| TFF3        | trefoil factor 3 (intestinal)            |
| CKS2        | CDC28 protein kinase regulatory subunit 2 |
| GDF15       | growth differentiation factor 15         |
| ASNS        | asparagine synthetase (glutamine-hydrolyzing) |
| DDIT3       | DNA-damage-inducible transcript 3        |
Table S7. Primer design for qRT-PCR validation experiment.

| Gene      | Roche TaqMan Probe | Forward primer             | Reverse primer             |
|-----------|--------------------|---------------------------|----------------------------|
| CPQ       | #74                | 5′-gtggagccaagaattcataa-3′ | 5′-atcgaagaggtcaccacca-3′  |
| PLVAP     | #73                | 5′-atcccttgaccccccactcc-3′ | 5′-tgagcatatccccctgctc-3′  |
| MAFB      | #82                | 5′-agggagctgccacgcctc-3′  | 5′-attggccattcaagcaagctg-3′|
| ACVRL1    | #71                | 5′-agacccccacccaactctca-3′ | 5′-cgcatctgactgctggtg-3′   |
| CLEC3B    | #40                | 5′-ctcaagagcgctctggacta-3′ | 5′-gcgtccctccagggcctac-3′  |
| DIP2B     | #8                 | 5′-ctctgctttaaatgcaccag-3′ | 5′-ttctctctgtctgtgctg-3′   |
| GABARAPL2 | #70                | 5′-ccctgctttgtgtgtgtc-3′  | 5′-ctctagctgctgtgctc-3′    |
| LIMK2     | #87                | 5′-tcactgtctcttctatgtc-3′ | 5′-tcccagcttgcagttgcag-3′  |
| ZFYVE21   | #22                | 5′-ggagctgctcgaggtaggtc-3′ | 5′-ccagttgcaggggctggtg-3′  |
| ZMYND11   | #42                | 5′-tgacattgctggaggtctat-3′ | 5′-caggagagcattgaccaag-3′  |
| EGR2      | #3                 | 5′-ttgatccaccaacctact-3′  | 5′-agacccacacttctggctac-3′ |
| DDIT3     | #9                 | 5′-cagaggtgtaacgtgagag-3′  | 5′-tggtatggctgtttttggtg-3′|
| ASNS      | #2                 | 5′-gattacactacagggttca-3′  | 5′-cactcctctctgctgctg-3′   |
| GDF15     | #28                | 5′-ccggatactcgcagggctta-3′ | 5′-agagtttctgcaagttgctc-3′ |
| SLC26A4   | #84                | 5′-ggagaagggaggaagaggg-3′  | 5′-caagagatggaggtagctc-3′  |
| FAM189A2  | #43                | 5′-cctgttacctctacttctct-3′ | 5′-actgcaagctacagctgtc-3′  |
| TFF3      | #4                 | 5′-ggagagacatgctgtgctg-3′  | 5′-gtggggaggtctgctgacta-3′ |
| CKS2      | #25                | 5′-ttgacgaacactacgagta-3′  | 5′-agctagactcgttgtggc-3′   |