Supplemental information

Targeting non-canonical pathways
as a strategy to modulate
the sodium iodide symporter

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Figure S1 (related to Fig. 1, 2G, 3C, 3D). Identification of FDA-Approved Drugs that Enhance NIS Function

(A) High throughput screen (HTS) based on a newly adapted YFP biosensor assay was used in the primary screen (green boxes) to determine the efficacy of 1200 drugs (Prestwick Chemical Library, single dose) to increase intracellular iodide in TPC1-NIS-YFP cells. A secondary multi-dose screen (orange boxes) was undertaken to validate 73 of these drugs at 10 different doses in two YFP-expressing cell lines (i.e., TPC-1-NIS-YFP and TPC1-YFP). From this, a total of 40 drugs were validated in radioiodide uptake assays (red box) in multiple cell types as listed. Finally, a shortlist of 10 drugs was generated (blue box) and different drug combinations evaluated in radioiodide uptake assays. (B) TPC-1-NIS-YFP cell viability following treatment with a 10 μM dose of 1200 drugs (Prestwick Chemical Library) for 24 hr. Each circle represents a mean value from 2 biological replicates. Table (left) summarizes number of drugs associated with TPC-1-NIS-YFP cellular viability equal or greater than 70, 80 or 90%. Cellular viability determined using the alamarBlue (resazurin) assay. (C) YFP-based biosensing and cell viability of TPC-1-NIS-YFP cells treated for 24 hr at 10 different drug doses (0.1 – 50 μM). 8 out of 73 representative drug profiles are shown. Blue circles represent mean ΔYFP values from 2 biological replicates. Cell viability (red circles) was determined using the alamarBlue (resazurin) assay (Mean ± S.E.M., n = 4). Pharmacologic parameters (e.g., AUC) were determined up to maximum drug dose associated with cell viability ≥ 70% (indicated by vertical black line). (D, E) Radioiodide uptake of TPC-1-NIS cells (D) and primary human thyrocytes (E) treated with the indicated drug for 24 hr. Data for astemizole† is compiled from human primary thyrocytes treated with two different doses (0.5 and 0.75 μM). (F) Western blot analysis of NIS expression in TPC-1-NIS cells treated with SAHA at indicated doses for 24 hr. (G) Radioiodide uptake of TPC-1-NIS cells treated with SAHA at indicated doses for 24 hr. (H) Western blot analyses of NIS protein levels in TPC-1-NIS (upper) and 8505C-NIS cells (lower) at indicated times post-treatment (hr) with 2.5 μM SAHA. (I) Same as (D) but radioiodide uptake in TPC-1-NIS cells at indicated times post-treatment (hr) with 2.5 μM SAHA. Data presented as mean ± S.E.M., n = 3, one-way ANOVA followed by Dunnett’s post hoc test (NS, not significant; *p < 0.05; **p < 0.01; ***p < 0.001) or unpaired two-tailed t-test (*p < 0.05).
Figure S2 (related to Fig. 3). Combined VCP Inhibitor and SAHA Treatment Augments Radioiodide Uptake in Thyroid and Breast Cancer Cells

(A, B) Radioiodide uptake (A) and relative NIS expression (B) in TPC-1-NIS cells treated with 2.5 μM eyarestatin-1 (ES-1) or 5.0 μM NMS-873 either alone or in combination with 2.5 μM SAHA. SAHA was added 12 hr prior to ES-1 or NMS-873. (C) Radioiodide uptake of MDA-MB-231-NIS cells treated with carebastine (CBT) at indicated doses for 24 hr. Controls: DMSO and 0.5 μM ebastine (EBT). (D) Relative NIS protein expression in parental TPC-1 cells treated with 0.2 μM selumetinib (SEL), 2.5 μM SAHA, 0.5 μM EBT and 1.0 μM CBT, either alone or in combination as indicated. SEL treatment included for comparison. (E) Relative VCP expression in parental TPC-1 cells treated with 2.5 μM SAHA and 1.0 μM CBT either alone or in combination. (F, G) Radioiodide uptake (F) and relative NIS mRNA levels (G) in parental MDA-MB-231 cells treated with 2.5 μM SAHA and 1.0 μM CBT either alone or in combination. (H) Same as (E) but with parental MDA-MB-231 cells. (I-K) Same as (F-H) but with parental MCF7 cells. (L) Same as (D) but with parental MCF7 cells. Red boxes highlight induction of NIS protein by SAHA and SAHA+CBT compared to DMSO or CBT treatment alone. (M) Relative VCP protein levels in TPC-1-NIS cells following VCP-siRNA depletion and treatment with 1.0 μM CBT and/or 2.5 μM SAHA. (N, O) Radioiodide uptake (N) and relative NIS mRNA levels (O) in MDA-MB-231-NIS cells following VCP-siRNA depletion and treatment with 1.0 μM CBT. (P, Q) Relative VCP mRNA (P) and protein levels (Q), as well as NIS expression (Q), in MDA-MB-231-NIS cells following VCP-siRNA depletion and treatment with 1.0 μM CBT and/or 2.5 μM SAHA. Data presented as mean ± S.E.M., n = 3-6, one-way ANOVA followed by Tukey’s (A, F, I) or Dunnett’s (C, M, N, O) post hoc test (NS, not significant; *p < 0.05; **p < 0.01; ***p < 0.001) or unpaired two-tailed t-test (*p < 0.05; ##p < 0.01; ###p < 0.001).
Figure S3 (related to Fig. 3L, 4A-E). Autophagy Inhibitor BafA1 Augments NIS Expression and Radioiodide Uptake in Thyroid Cells

(A) Scanning densitometry performed relative to β-actin on Western blot analysis of LC3B-II (left) and p62 protein levels (right) in TPC-1-NIS cells treated with 1.0 μM carebastine (CBT, red spots) or 100 nM bafilomycin A1 (bafA1, blue spots) for the indicated timecourse (hr). See also Figure 3L. (B) Western blot analysis of LC3B-I, LC3B-II and p62 protein levels in 8505C-NIS cells treated with 1.0 μM CBT (left) or 100 nM bafA1 (right) for the indicated timecourse (hr). Scanning densitometry (below) performed relative to β-actin. Relative NIS expression levels are also shown for CBT treated 8505-NIS cells. (C-D) Western blot analysis of NIS protein levels in TPC-1-NIS and 8505C-NIS cells treated with 100 nM bafA1 for the indicated timecourse (hr). Scanning densitometry performed relative to β-actin (D). (E) Radioiodide uptake of primary human thyrocytes treated with bafA1 at the indicated dose for 24 hr. (F) Radioiodide uptake of TPC-1-NIS and 8505C-NIS cells treated with 0.5 μM disulfiram for the indicated timecourse. (G) Relative NIS protein levels in TPC-1-NIS and 8505C-NIS cells treated with 0.5 μM disulfiram for the indicated timecourse (hr). Scanning densitometry (right) performed relative to β-actin (n = 6). (H) Western blot analysis of LC3B-I, LC3B-II and p62 protein levels in TPC-1-NIS and 8505C-NIS cells treated with 0.5 μM disulfiram for the indicated timecourse (hr). Scanning densitometry (below) performed relative to β-actin (n = 3). (I) Evaluation of VCP binding to NIS using NanoBiT in HeLa cells treated with disulfiram or VCP inhibitor CB5083 for 24 hr at the indicated dose (left, n = 4). Normalised NanoBiT assay results shown at 6 min after addition of Nano-Glo live cell assay solution (right). (J) Radioiodide uptake of 8505C-NIS cells following VCP-siRNA depletion and treatment with 0.5 μM disulfiram. (K) Relative NIS and VCP protein levels in 8505C-NIS cells following VCP-siRNA depletion and treatment with 0.5 μM disulfiram. Data presented as mean ± S.E.M., one-way ANOVA followed by Dunnett’s post hoc test (NS, not significant; *p < 0.05; **p < 0.01; ***p < 0.001) or unpaired two-tailed t-test (⁎p < 0.05; ⁎⁎p < 0.01; ⁎⁎⁎p < 0.01).
**Figure S4 (related to Fig. 5A-C). Identification of Proteostasis Modulators Associated with Cancer Genetic Signature, Recurrence and Radioiodide Therapy**

(A) (upper) Volcano plots comparing log2FC with q-value (-log base 10) for 142 core proteostasis genes in the BRAF-like (left) or RAS-like (middle) THCA cohort (C) versus normal (N). (upper right) Volcano plot illustrating log2FC with q-value (-log base 10) for 142 core proteostasis genes in the RAI-treated THCA cohort [recurrent (REC) versus non-recurrent (NON-REC)]. (lower) Volcano plots comparing log2FC with q-value (-log base 10) for 142 core proteostasis genes in the BRAF-like (left), RAS-like (middle) or entire (right) THCA cohort [REC versus NON-REC]. Gene categories indicated by coloured spots. (B) Box and whisker plots showing expression (log2) of 13 core proteostasis genes in the BRAF-like, RAI-treated THCA cohort [recurrent (REC) versus non-recurrent (NON-REC)]; P-values determined by Mann-Whitney test and adjusted using the Benjamini-Hochberg FDR correction procedure (NS, not significant; *p < 0.05; **p < 0.01; ***p < 0.001). (C) Stacked bar charts showing clinical characteristics of (i) RAI (n = 260) versus non-RAI (n = 173) treated THCA patients or (ii) THCA patients stratified by BRAF-like (n = 272) or RAS-like (n = 119) tumoral genetic signatures. Clinical staging attributes include risk group, tumor staging, lymph node staging and disease staging. P-values derived using Chi-Squared test and adjusted using the Benjamini-Hochberg FDR correction procedure. NS, not significant.
### C. THCA (BRAF-like, RAI treated (n = 137)): ROC analysis cut-off values

#### ATG2A: High vs. Low
- Sensitivity: 0.629, Specificity: 0.686, AUC: 0.686, p = 0.009
- Sensitivity: 0.576, Specificity: 0.670, AUC: 0.726, p = 0.001

#### PSMII: High vs. Low
- Sensitivity: 0.666, Specificity: 0.686, AUC: 0.726, p = 0.009
- Sensitivity: 0.576, Specificity: 0.670, AUC: 0.726, p = 0.001

#### VCP: High vs. Low
- Sensitivity: 0.629, Specificity: 0.686, AUC: 0.686, p = 0.009
- Sensitivity: 0.576, Specificity: 0.670, AUC: 0.726, p = 0.001

### D. THCA (BRAF-like, RAI-treated, n = 137): Percentile cut-off values

| Gene     | 90th percentile | 95th percentile | 99th percentile | 99.5th percentile | 99.9th percentile |
|----------|-----------------|-----------------|-----------------|-------------------|------------------|
| APD1     | 100             | 100             | 100             | 100               | 100              |
| AP4B1    | 100             | 100             | 100             | 100               | 100              |
| ATG2A    | 100             | 100             | 100             | 100               | 100              |
| ATG9A    | 100             | 100             | 100             | 100               | 100              |
| BECN1    | 100             | 100             | 100             | 100               | 100              |
| HPS1     | 100             | 100             | 100             | 100               | 100              |
| HPSA5    | 100             | 100             | 100             | 100               | 100              |
| PSMD2    | 100             | 100             | 100             | 100               | 100              |
| PSMD8    | 100             | 100             | 100             | 100               | 100              |
| PSMD11   | 100             | 100             | 100             | 100               | 100              |
| SQSTM1   | 100             | 100             | 100             | 100               | 100              |
| VCP      | 100             | 100             | 100             | 100               | 100              |

**Genes (p < 0.05; q < 0.05):**
- ATG2A
- ATG9A
- BECN1
- HPS1
- HPSA5
- PSMD2
- PSMD8
- PSMD11
- SQSTM1
- VCP
Figure S5 (related to Fig. 5D-F). A 13 Proteostasis Gene Panel is Associated with Recurrence in the BRAF-like, RAI-Treated THCA Cohort

(A) Representative ROC curves of 6 proteostasis genes in the BRAF-like, RAI-treated THCA cohort (n = 137). (B) Comparison of clinical sensitivity and specificity of 13 proteostasis genes derived by ROC analysis using optimal cut-off expression values (log2 values) in the BRAF-like, RAI-treated THCA cohort (n = 137). (C) Representative Kaplan-Meier analysis of DFS for the BRAF-like, RAI-treated THCA cohort stratified on high versus low tumoral expression of indicated core proteostasis genes; log-rank test. Number (n) of patients per expression sub-group (high/low), p-values and q-values are shown. (D) Kaplan-Meier analysis of the BRAF-like, RAI-treated THCA cohort stratified on high versus low tumoral expression according to indicated percentile cut-off values; log-rank test. Significance indicated by p- and q-values. \( \text{N}_{\text{REC}} \) = number of recurrent cases in cohort with either high (left) or low (right) tumoral proteostasis expression. Green = FDR < 5%; Orange = greatest level of significance per percentile cut-off group; Yellow = highest percentage of stratified recurrent cases per percentile cut-off group.
A  THCA (BRAF-like, RAI-treated, n = 137)

| Gene      | Multivariate Coefficient | p-value |
|-----------|--------------------------|---------|
| AP3D1    | 0.00000882               | 0.982   |
| AP4E1    | 0.0002902                | 0.584   |
| ATG2A    | -0.009032                | 0.447   |
| ATG5A    | 0.0001311                | 0.116   |
| BECN1    | 0.000286                  | 0.110   |
| HSPH1    | -0.000125                | 0.831   |
| HSPH2    | 0.0000045                | 0.969   |
| PSMD2    | -0.001065                | 0.687   |
| PSMD8    | 0.000185                 | 0.687   |
| PSMD11   | 0.000249                 | 0.440   |
| SEC24C   | 0.0002052                | 0.281   |
| SQSTM1   | 0.000036                 | 0.653   |
| VCP      | 0.000265                 | 0.354   |

B  THCA (BRAF-like, RAI-treated, n = 137)

| RiskScore | Sensitivity (%) | Specificity (%) | HR (95% CI) | p-value |
|-----------|-----------------|-----------------|-------------|---------|
| 10.84     | 190             | 18.97           | 26.381 (0.164-4231.231) | NS      |
| 12.69     | 95.24           | 69.83           | 35.862 (4.81-267.405)   | 4.79x10^-4 |
| 12.73     | 90.48           | 71.55           | 17.794 (4.142-76.445)   | 1.09x10^-3 |
| 12.86     | 85.71           | 75.86           | 13.352 (3.930-45.358)   | 3.3x10^-3  |
| 13.03     | 89.95           | 80.17           | 11.978 (4.027-35.626)   | 8.0x10^-6  |
| 13.13     | 71.43           | 82.76           | 7.977 (3.090-20.589)    | 1.8x10^-5  |
| 13.16     | 66.67           | 82.76           | 6.980 (2.812-17.326)    | 2.8x10^-5  |
| 13.21     | 61.9            | 83.62           | 6.189 (2.554-14.951)    | 5.1x10^-5  |

C  GSE27155: PTC (BRAF T1799A, n = 26) v N (n = 4)

D  GSE27155: PTC (RAS mutation, n = 7) v N (n = 4)

E  GSE27155

F  GSE27155 v THCA

142 proteostasis gene panel

- UPR
- Proteasomal
- Autophagy
- Transport
- Not significant

GSE27155 (BRAF T1799A PTC v N: 58/129 proteostasis genes; p < 0.05; FDR < 10%)

THCA (BRAF-like PTC v N: 102/142 proteostasis genes; p < 0.03; FDR < 5%)

**p = 0.032**
Figure S6 (related to Fig. 5C, G, H, I). Profiling and Validation of the 13 Proteostasis Gene Riskscore Classifier

(A) Regression coefficients of 13 proteostasis genes in the BRAF-like, RAI-treated THCA cohort (multivariate Cox regression analysis). (B) Profile of riskscore cut-off values of the 13 gene proteostasis riskscore classifier along with estimations of sensitivity and specificity (%) for the BRAF-like, RAI-treated THCA cohort (n = 137). Hazard ratios (HR) ± 95% CI using riskscore cut-off values to stratify into high and low risk cohorts are shown (univariate Cox regression analysis). (C, D) Volcano plot comparing log2FC with q-value (-log base 10) for 142 core proteostasis genes in the GSE27155 dataset [BRAF T1799A PTC versus N (C) and RAS mutation PTC versus N (D)]. Gene categories indicated by coloured spots. (E) Representative box and whisker plots showing expression (log2) of 5 core proteostasis genes in the GSE27155 (BRAF T1799A PTC versus normal; left) and THCA (BRAF-like PTC versus normal; right) datasets; P-values determined by Mann-Whitney test and adjusted using the Benjamini-Hochberg FDR correction procedure (**p < 0.01; ***p < 0.001). (F) Venn diagram showing significant overlap in the 142 core proteostasis gene panel between the GSE27155 (BRAF T1799A PTC versus normal) and THCA (BRAF-like PTC versus normal) datasets. Genes in the 13 gene proteostasis riskscore classifier are underlined.
Figure S7 (related to Fig. 5). Greater Predictive Value of the 13-Gene Riskscore Classifier Compared to a Panel of Molecular Biomarkers

(A) Volcano plot comparing log2FC with q-value (-log base 10) for 142 core proteostasis genes in the GSE276039 dataset [anaplastic thyroid cancer (n = 17) versus PDTC (n = 12)]. (B) Volcano plot illustrating log2FC with q-value (-log base 10) for 142 core proteostasis genes in the entire THCA cohort [T3+T4 versus T1+T2 (middle); N1 versus N0 (right)]. Gene categories indicated by coloured spots. (C) Venn diagram showing overlap in the 142 core proteostasis gene panel between the GSE276039 (anaplastic thyroid cancer versus PDTC), THCA (T3+T4 versus T1+T2) and THCA (N0 versus N1) datasets. Genes in the 13 gene proteostasis risk score classifier are underlined. (D) Representative ROC curves (left) of 3 molecular biomarkers in the BRAF-like, RAI-treated THCA cohort (n = 137). Comparison of ROC analysis (right) using 7 molecular biomarkers in the BRAF-like, RAI-treated THCA cohort (n = 137). AUC, p-values and optimal cut-off expression values (log2 values) are shown. (E) Representative Kaplan-Meier analysis of DFS for the BRAF-like, RAI-treated THCA cohort (n = 124) stratified on high versus low tumoral expression of 7 genes reported as biomarkers of thyroid cancer recurrence; log-rank test. Kaplan-Meier analysis using the 13-gene riskscore classifier to stratify patients is included for comparison. Number (n) of patients per expression/risk sub-group (high/low) and p-values are shown.
Table S1 (related to Fig. 1D, 1E, S1A, S1B). Top 50 Drugs Identified in the Primary High Throughput Screen Using YFP as a Biosensor of Intracellular Iodide

| Drug                         | ΔYFP  | Cell Viability (%) | Category                        | Known or putative target(s)                          |
|------------------------------|-------|--------------------|---------------------------------|------------------------------------------------------|
| Antimycin A                   | 5.063 | 89.30              | Phagocytosis                     | Autophagy                                            |
| Dexamethasium                 | 5.008 | 77.22              | Anticholinesterase              | N/A                                                  |
| Ethastine                     | 4.524 | 83.37              | Antihistamine                    | VCP                                                  |
| Tiacomazole                   | 4.254 | 61.72              | Antifungal                      | Autophagy (ATG4B inhibitor)                         |
| Bromocryptine                 | 4.199 | 51.41              | Dopamine agonist                 | Autophagy                                            |
| Vanoverine                    | 4.145 | 64.00              | Dopamine reuptake                | N/A                                                  |
| Dequalinium                   | 3.757 | 54.66              | Antimicrobial                    | N/A                                                  |
| Articaine                     | 3.598 | 84.90              | Anesthetic                       | N/A                                                  |
| Astemizole                    | 3.259 | 9.85               | Antifungal                       | Lysozymotrophic, VCP                                 |
| Nomegestrol                   | 3.189 | 99.87              | Progestogen                      | N/A                                                  |
| Eucatropine                   | 3.087 | 96.65              | Anticholinergic                  | N/A                                                  |
| Lithocholic acid              | 3.078 | 77.97              | Detergent                        | Autophagy, ER stress                                 |
| Tofloxacacin                  | 2.959 | 76.29              | Antibiotic                       | N/A                                                  |
| Clofibrate                    | 2.923 | 89.98              | Antifungal                       | VCP                                                  |
| Rosiglitazone                 | 2.854 | 96.47              | Hypoglycemic                     | Autophagy, mTOR                                      |
| Vatalanib                     | 2.832 | 78.49              | Antinecancer                     | Tyrosine kinase inhibitor (TK1)                      |
| Pyridium                      | 2.831 | 72.32              | Anthelmintic                     | Autophagy                                            |
| Phenolamine                   | 2.830 | 108.85             | o-adrenergic antagonist          | N/A                                                  |
| Atenolol                      | 2.808 | 98.52              | Beta blocker                     | N/A                                                  |
| Iprapride                     | 2.760 | 80.40              | Dopamine D2 antagonist           | N/A                                                  |
| Formoterol                    | 2.741 | 119.65             | β2(2)-agonist                    | Autophagy, proteasomal                               |
| Fenofibrate                   | 2.606 | 103.58             | Lipid-lowering                   | Autophagy                                            |
| Terfenadine                   | 2.578 | 83.49              | Antihistamine                    | VCP                                                  |
| Clofibrate                    | 2.534 | 39.77              | Anti-arrhythmia                  | N/A                                                  |
| Rivastigmine                  | 2.483 | 122.32             | Anticholinesterase              | N/A                                                  |
| Pravastatin                   | 2.467 | 101.97             | Lipid-lowering                   | HMG-CoA reductase                                    |
| Fenofibrate                   | 2.412 | 70.95              | Calcium channel blocker          | N/A                                                  |
| Imatinib                      | 2.358 | 105.36             | Antinecancer                     | Autophagy, lysosomotrophic, TKI                      |
| Mifepristone                  | 2.341 | 93.41              | Antiprogestogenic                | Autophagy, unfolded protein response                 |
| Pisciprazole                  | 2.321 | 85.25              | Antipsychotic                    | N/A                                                  |
| Methyldopate                  | 2.311 | 103.17             | Antihypertensive                 | N/A                                                  |
| Hesperidin                    | 2.270 | 68.98              | Flavonol glycoside              | Autophagy, ER stress                                 |
| Rufloxacin                    | 2.269 | 112.38             | Antibiotic                       | N/A                                                  |
| Nortriptiline                 | 2.248 | 74.00              | Antidepressant                   | Autophagy, lysosomotrophic                           |
| Methotrexaproprazine          | 2.222 | 91.01              | Phenothiazine                    | N/A                                                  |
| Oxyphenbutazone               | 2.219 | 62.83              | Anti-inflammatory                | N/A                                                  |
| Prednisolone                  | 2.209 | 92.22              | Anti-inflammatory                | N/A                                                  |
| Phenformin                    | 2.165 | 102.43             | Antidiabetic                     | Autophagy, mTOR                                      |
| Clozapine                     | 2.151 | 87.88              | Antidepressant                   | Autophagy, lysosomotrophic                           |
| Halofantrine                  | 2.143 | 105.63             | Antimalarial                     | Autophagy, lysosomotrophic                           |
| Sisomicin                     | 2.122 | 147.72             | Antibiotic                       | N/A                                                  |
| Oxatol                       | 2.115 | 78.93              | Anthelmintic                     | N/A                                                  |
| Trimethoprim                  | 2.108 | 97.82              | Antibiotic                       | N/A                                                  |
| Chloroquine                   | 2.075 | 120.91             | Antimalarial                     | Autophagy, lysosomotrophic, vesicular transport      |
| Propylfluorid                 | 2.070 | 85.28              | Intercalation                    | N/A                                                  |
| Meclozine                     | 2.060 | 69.82              | Anti-Histamine                   | Histamine II antagonist                              |
| Quinidine                     | 2.059 | 88.15              | Anti-arrhythmia                  | Lysosomotrophic                                     |
| Ceforanide                    | 2.049 | 89.90              | Antibiotic                       | N/A                                                  |
| Cimoxin                      | 2.009 | 120.13             | Anti-inflammatory                | N/A                                                  |
| Tropisetron                   | 2.004 | 78.57              | Antiemetic                       | N/A                                                  |

Drugs listed in order of ΔYFP values utilizing TPC-1-NIS cells treated with a 10 μM dose for 24 hr. TPC-1-NIS cell viability (%) after 24 hr drug treatment, drug category and known or putative proteostasis drug targets are shown.
### A TPC-1-NIS-YFP cells (> 70% cell viability)

| Drug                      | AUC | Maximal ΔYFP | Dose (μM) | Drug                      | AUC | Maximal ΔYFP | Dose (μM) | Drug                      | AUC | Maximal ΔYFP | Dose (μM) |
|---------------------------|-----|--------------|-----------|---------------------------|-----|--------------|-----------|---------------------------|-----|--------------|-----------|
| Demeclarium               | 8.02| 5.06         | 25        | Alverine                  | 3.63| 2.52         | 12.50      | Levocabastine             | 1.15| 2.10         | 3.13      |
| Pyrinizine                | 6.73| 6.17         | 0.78      | Clofibrate                | 3.32| 2.53         | 0.20       | Amikacin                  | 0.65| 2.29         | 0.20      |
| Guanabenz                 | 5.56| 4.12         | 1.56      | Phenformin                | 3.19| 3.97         | 50         | Sertaconazole             | 0.36| 2.44         | 25        |
| Propargacine              | 5.41| 3.87         | 0.39      | Sulconazole               | 3.09| 3.83         | 12.5       | (R)-Atenolol              | 3.16| 1.87         | 0.78      |
| Niflumic acid             | 4.95| 2.70         | 1.56      | Terfenadine               | 3.00| 2.56         | 6.25       | Azathioprine              | 2.93| 1.92         | 12.50     |
| Morocidine                | 4.86| 2.61         | 0.39      | Halofantrine              | 2.28| 2.06         | 3.13       | Flornicolide              | 2.61| 1.74         | 0.10      |
| Nortriptyline             | 4.46| 2.52         | 12.50     | Hexachlorophene           | 2.00| 3.10         | 50         | Zimelidine                | 2.23| 1.94         | 6.25      |
| Chlorhexitine             | 4.24| 5.09         | 6.25      | Retinonic acid            | 1.62| 2.91         | 0.39       | Amrinone                  | 2.11| 1.91         | 3.13      |
| Ebastine                  | 4.19| 4.52         | 6.25      | Disulfiram                | 1.55| 2.78         | 3.13       | Praziquantel              | 1.83| 1.71         | 12.5      |
| Ethacrynic acid           | 3.70| 2.22         | 0.10      | Hesperidin                | 1.15| 2.76         | 0.20       | Articaine                 | 1.22| 1.66         | 1.56      |

### B TPC-1-YFP cells (> 70% cell viability)

| Drug                        | AUC | Maximal ΔYFP | Dose (μM) | Drug                        | AUC | Maximal ΔYFP | Dose (μM) |
|-----------------------------|-----|--------------|-----------|-----------------------------|-----|--------------|-----------|
| Prednisolone                | 4.95| 3.27         | 0.78      | Methotreximeprazone         | 1.38| 2.92         | 50        |
| Pirepazolazine              | 3.81| 3.02         | 12.5      | Guanabenz                  | 2.40| 1.94         | 3.13      |
| Demeclarium                 | 3.50| 4.51         | 12.5      | Fenofibrate                | 2.25| 1.59         | 12.5      |
| Pyrinizine                  | 3.37| 4.99         | 0.78      | Praziquantel               | 2.22| 1.91         | 0.39      |
| Phenformin                  | 3.31| 3.90         | 50        | Piretanide                 | 1.41| 1.86         | 0.1       |
| Miosartine                  | 3.30| 4.34         | 50        | Niflumic acid              | 1.26| 1.87         | 0.39      |
| Sertaconazole              | 3.05| 3.57         | 25        | Tioconazole                | 1.15| 1.54         | 0.20      |
| Ebastine                    | 2.94| 4.56         | 6.25      | Tridihexethyl              | 0.92| 1.92         | 50        |
| Dexamethasone               | 2.24| 2.03         | 0.78      | Retinonic acid             | 0.83| 1.89         | 0.39      |
| Chlorhexitine               | 2.17| 2.49         | 6.25      | Clomiphene                 | 0.82| 1.81         | 12.5      |
| Ethacrynic acid             | 1.72| 2.02         | 0.10      | Clomipramine               | 0.78| 1.67         | 25        |
| Disulfiram                  | 1.69| 2.55         | 0.39      | Vanoxerine                 | 0.73| 1.64         | 6.25      |
| Oximidine                   | 1.58| 2.41         | 12.5      | Bisacodyl                  | 0.42| 1.86         | 50        |

### C TPC-1-NIS-YFP v TPC-1-YFP cells (> 70% cell viability; i.e. adjusted for YFP effects)

| Drug                      | ΔAUC | Drug                      | ΔAUC | Drug                      | ΔAUC |
|---------------------------|------|---------------------------|------|---------------------------|------|
| Propargacine              | 3.31 | Sulconazole               | 3.06 | Hesperidin                | 1.14 |
| Morocidine                | 4.57 | Azathioprine              | 2.86 | Levocabastine             | 1.13 |
| Demeclarium               | 4.53 | Clofibric acid            | 2.81 | Halofantrine              | 1.06 |
| Nortriptyline             | 4.24 | Terfenadine               | 2.53 | Articaine                 | 0.95 |
| Niflumic acid             | 3.70 | Chlorhexitine             | 2.07 | Ketamine                  | 0.92 |
| Alverine                  | 3.45 | Hexachlorophene           | 2.00 | Retinolic acid            | 0.70 |
| Pyrinizine                | 3.36 | Ethacrynic acid           | 1.96 | Quinidine                 | 0.68 |
| Clofibric acid            | 3.32 | Zimelidine                | 1.81 | Molindone                 | 0.66 |
| Guanabenz                 | 3.16 | Amrinone                  | 1.77 | Atenolone                 | 0.60 |
| (R)-Atenolol              | 3.16 | Hesperidin                | 1.25 | Amikacin                  | 0.54 |

### D TPC-1-NIS-YFP

- **Maximal ΔYFP**
- **AUC**

### E TPC-1-NIS-YFP (○) vs TPC-1-YFP (●)

- **Cumulative fraction**
- **AUC**
- **P = 0.0001**
Table S2 (related to Fig. 1F, S1C). Ranking of Drug Efficacy to Enhance Intracellular Iodide Adjusting for Multiple Doses, YFP-Only Effects, and Cell Viability

(A) Top 30 drugs ranked on area under the curve (AUC) values derived from ΔYFP values of TPC-1-NIS-YFP cells treated with multiple drug doses (0.1-50 μM) and > 70% cell viability. Maximal ΔYFP value and associated dose (μM) for each drug are shown. Drugs with maximal ΔYFP values < 2 were ranked lower. (B) Same as (A) but with TPC-1-YFP cells. (C) Top 30 drugs ranked on ΔAUC values, i.e., difference in AUC values between drug-treated TPC-1-NIS-YFP and TPC-1-YFP cells. (D) Representative dose response YFP-iodide profile highlighting pharmacologic parameters used in analysis, i.e., area under the curve (AUC) and maximal ΔYFP values, in TPC-1-NIS-YFP cells treated with niflumic acid. (E) Comparison of drug efficacy in thyroid cells with different NIS levels. Cumulative frequency distribution plot comparing AUC values for top 30 drugs identified to increase intracellular iodide in parental TPC-1-YFP and TPC-1-NIS-YFP cells; P-value determined by the Kolmogorov-Smirnov test.
Table S3 (related to Fig. 3A, Table 1). Chemical Structure of Putative VCP inhibitors

| Drug          | 2D Structure | 3D Structure | Supporting Evidence                                                                 |
|---------------|--------------|--------------|------------------------------------------------------------------------------------|
| Carebastine   | ![2D Image](image1) | ![3D Image](image2) | Drug Combination, RAI Uptake & VCP siRNA assays (this study)                       |
|               |              |              | Active carboxylic acid metabolite of ebastine                                      |
| Fexofenadine  | ![2D Image](image3) | ![3D Image](image4) | RAI Uptake assays (this study)                                                     |
|               |              |              | Active metabolite of terfenadine                                                  |
| Terfenadine   | ![2D Image](image5) | ![3D Image](image6) | CMAP (L1000) & RAI Uptake assays (this study)                                      |
|               |              |              | CMAP (build 02): MCF7 Breast cells (p = 3.0E-31) (Segura-Cabrera et al., 2017)   |
| Disulfiram    | ![2D Image](image7) | ![3D Image](image8) | Drug Combination & RAI Uptake assays (this study)                                  |
|               |              |              | Disulfiram Metabolite (DTC) Binds NPL4 to Disable the VCP-NPL4-UF1 Pathway (Skrott et al., 2017) |
| Ebastine      | ![2D Image](image9) | ![3D Image](image10) | Drug Combination & RAI Uptake assays (this study)                                  |
|               |              |              | Structure-Based Virtual Screening, Cell-Based & Biochemical assays (Segura-Cabrera et al., 2017) |
|               |              |              | RAI Uptake, VCP siRNA & Cell Surface Biotinylation (CSB) assays (Fletcher et al., 2020) |
| Astemizole    | ![2D Image](image11) | ![3D Image](image12) | Drug Combination & RAI Uptake assays (this study)                                  |
|               |              |              | CMAP (build 02): MCF7 Breast cells (p = 8.4E-39) Structure-based Virtual Screening, Cell-Based & Biochemical assays (Segura-Cabrera et al., 2017) |
|               |              |              | RAI uptake, VCP siRNA & CSB assays (Fletcher et al., 2020)                         |
| Ciotrimazole  | ![2D Image](image13) | ![3D Image](image14) | CMAP (L1000), Drug Combination & RAI Uptake assays (this study)                   |
|               |              |              | CMAP (build 02): MCF7 Breast cells (p = 4.0E-46) Structure-Based Virtual Screening Cell-Based & Biochemical assays (Segura-Cabrera et al., 2017) |
|               |              |              | RAI uptake, VCP siRNA & CSB assays (Fletcher et al., 2020)                         |
| NMS-873       | ![2D Image](image15) | ![3D Image](image16) | Well-established VCP inhibitor                                                    |
|               |              |              | Used as a control drug for VCP inhibitor studies                                   |
|               |              |              | Drug combination & RAI uptake assays (this study)                                 |

2D and 3D images of putative VCP inhibitors are shown, as well as supporting evidence for their ability to target VCP function and enhance NIS function to increase radioiodide uptake. Source: ChemSpider; PubChem.
Table S4 (related to Fig. 5, S4, S6, S7). Panel of 142 Core Proteostasis Genes

| Unfolded Protein Response (UPR) | Proteasomal | Autophagy | Transport (protein, vesicular) |
|---|---|---|---|
| ATF4 | PSMA1 | PSMC1 | PSMD12 | ATG2A | EPG5 | APIAR | APIM2 | COPG1 | SEC23A |
| ATF6 | PSMA2 | PSMC2 | PSMD3 | ATG2B | ERN1 | APIB1 | APIS1 | COPG2 | SEC23B |
| DDIT3 (CHOP) | PSMA3 | PSMC3 | PSMD4 | ATG3 | GABARAFLI (ATG8) | APIG1 | APIS2 | COPZ1 | SEC24A |
| EIF2AK3 (PERK) | PSMA4 | PSMC4 | PSME1 | ATG4A | SQSTM1 (p62) | APIG2 | AP4B1 | COPZ2 | SEC24B |
| HSPA5 (GRP78) | PSMA5 | PSMC5 | PSME2 | ATG4B | ULK1 (ATG1) | APIM1 | APIE1 | DNM2 | SEC24C |
| XBP1 | PSMA6 | PSMC6 | PSME3 | ATG4C | WDR45 | APIM2 | APIE1 | DTMNP1 | SEC24D |
| | PSMA7 | PSMD1 | PSME4 | ATG4D | WDR45B | API51 | ARCNI | EHD1 | SEC31A |
| | PSMB1 | PSMD2 | PSMF1 | ATG5 | WIP1 (ATG18) | API52 | BLOC1S3 | EHD2 | SEC31B |
| | PSMB2 | PSMD3 | UBB | ATG7 | WIP12 (ATG18B) | API53 | CAV1 | EHD4 | SNAP29 |
| | PSMB3 | PSMD4 | UBC | ATG9A | | AP2A1 | CAV2 | HPS1 | VAMP1 |
| | PSMB4 | PSMD5 | VCP | ATG10 | | AP2A2 | CLTA | HPS3 | VAMP2 |
| | PSMB5 | PSMD6 | ATG12 | | AP2B1 | CLTB | HPS4 | VAMP3 |
| | PSMB6 | PSMD7 | ATG13 | | AP2M1 | CLTC | HPS5 | VPS33B |
| | PSMB7 | PSMD8 | ATG14 | | AP2S1 | COPA | HPS6 | VPS33B |
| | PSMB8 | PSMD9 | ATG16L1 | | AP3B1 | COPB1 | PLIN3 | |
| | PSMB9 | PSMD10 | ATG16L2 | | AP3D1 | COPB2 | SARA1A | |
| | PSMB10 | PSMD11 | Becn1 (ATG6) | | AP3M1 | COPE | SEC13 | |

Functional categories of core proteostasis genes used in study which include: the unfolded protein response (UPR, 6 genes, orange), proteasomal degradation (45 genes, red), autophagy (26 genes, green) and transport (protein/vesicular, 65 genes, blue).
Table S5 (related to Fig. 5F). Core Proteostasis Genes are Predictive Indicators of an Increased Risk of Recurrence

| Gene     | Category | High vs Low Expression Groups | Cut-Off Value | B     | Hazard Ratio | 95.0% CI Lower | 95.0% CI Upper | p-value |
|----------|----------|-------------------------------|---------------|-------|--------------|----------------|----------------|---------|
| AP3D1    | Transport|                               | 11.89         | 1.311 | 3.709        | 1.574          | 8.741          | 0.003   |
| AP4B1    | Transport|                               | 8.23          | -1.620| 0.198        | 0.058          | 0.672          | 0.009   |
| ATG2A    | Autophagy|                               | 10.10         | 1.747 | 5.736        | 1.928          | 17.063         | 0.002   |
| ATG9A    | Autophagy|                               | 10.48         | 1.414 | 4.111        | 1.505          | 11.224         | 0.006   |
| BECN1    | Autophagy|                               | 10.76         | 1.065 | 2.901        | 1.222          | 6.892          | 0.016   |
| HPS1     | Transport|                               | 10.75         | 1.481 | 4.399        | 1.611          | 12.010         | 0.004   |
| HSPA5    | UPR      |                               | 14.04         | -1.437| 0.238        | 0.08           | 0.707          | 0.010   |
| PSMD2    | Proteasomal|                            | 11.76         | 1.221 | 3.390        | 1.241          | 9.256          | 0.017   |
| PSMD8    | Proteasomal|                            | 11.47         | 0.892 | 2.441        | 1.029          | 5.794          | 0.043   |
| PSMD11   | Proteasomal|                            | 9.48          | 1.591 | 4.908        | 1.650          | 14.601         | 0.004   |
| SEC24C   | Transport|                               | 11.52         | 1.414 | 4.112        | 1.506          | 11.228         | 0.006   |
| SQSTM1   | Autophagy|                               | 14.15         | 1.179 | 3.251        | 1.191          | 8.876          | 0.021   |
| VCP      | Proteasomal|                             | 12.66         | 2.374 | 10.744       | 1.442          | 80.073         | 0.021   |

Univariate Cox regression analysis in the BRAF-like, RAI-treated THCA cohort stratified using optimal expression cut-off values for 13 proteostasis genes. Cut-off value- log2 expression value; B- regression coefficient.
Table S6 (related to Fig. 5H, I). Univariate and Multivariate Analysis of the RAI-Treated (BRAF-like) and Non-RAI Treated THCA Cohort

### A

| Clinical Variable | BRAF-like, RAI-treated (n = 124) | Non-RAI treated (n = 151) |
|-------------------|----------------------------------|----------------------------|
|                   | Univariate                       | Multivariate               | Univariate                       | Multivariate               |
|                   | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) |
| **Age, years (±SD)** |         |              |         |              |         |              |         |              |
| < 50 | 79     | 0.072 | 2.288 (0.929-5.634) | 0.403 | 2.054 (0.380-11.105) | 83 | 0.057 | 1.159 (0.204-5.744) | 0.548 | 0.567 (0.089-3.604) |
| > 50 | 45     |         |              |         |              | 68 |         |              |         |
| **Gender** |         |              |         |              |         |              |         |              |
| Male | 39     | 0.994 | 0.996 (0.378-2.623) | 0.807 | 0.877 (0.308-2.500) | 28 | 0.300 | 2.456 (0.449-13.439) | 0.216 | 3.116 (0.516-18.826) |
| Female | 85    |         |              |         |              | 123 |         |              |         |
| **Stage** |         |              |         |              |         |              |         |              |
| I + II | 70     | 0.114 | 2.085 (0.839-5.185) | 0.856 | 0.855 (0.158-4.634) | 125 | 0.244 | 2.744 (0.502-15.004) | 0.677 | 2.104 (0.063-69.879) |
| III + IV | 54    |         |              |         |              | 26 |         |              |         |
| **T stage** |         |              |         |              |         |              |         |              |
| T1 + T2 | 62     | 0.222 | 1.789 (0.704-4.544) | 0.785 | 1.149 (0.424-3.113) | 120 | 0.403 | 2.663 (0.378-11.272) | 0.533 | 2.677 (0.104-79.966) |
| T3 + T4 | 62     |         |              |         |              | 31 |         |              |         |
| **Node stage** |         |              |         |              |         |              |         |              |
| N0 | 34     | 0.449 | 1.531 (0.508-4.617) | 0.109 | 2.587 (0.810-8.264) | 110 | 0.642 | 0.600 (0.070-5.147) | 0.412 | 0.396 (0.044-3.607) |
| N1 | 90     |         |              |         |              | 41 |         |              |         |
| **Risk score (13 gene classifier)** |         |              |         |              |         |              |         |              |
| High | 50     | 7.0x10^-4 | 32.614 (4.350-244.534) |         |              | 33 | 0.445 | 0.035 (0.000-190.755) | 0.974 | N/A |
| Low | 74     |         |              |         |              | 118 |         |              |         |

- **n**, number; **HR**, hazard ratio; **CI**, confidence interval. **P-values** in bold were less than 0.05 and considered statistically significant. Some patients in the BRAF-like, RAI-treated (n = 13) and non-RAI treated cohorts (n = 16) were not included in univariate and multivariate analysis of the THCA dataset due to missing clinical variables. 

### B

| Clinical Variable | BRAF-like, RAI-treated (n = 124) | Non-RAI treated (n = 151) |
|-------------------|----------------------------------|----------------------------|
|                   | Univariate                       | Multivariate               | Univariate                       | Multivariate               |
|                   | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) |
| **VCP expression** |         |              |         |              |         |              |         |              |
| High | 84     | 0.031 | 9.166 (1.223-68.679) | 0.038 | 8.472 (1.122-63.969) | 51 | 0.940 | 1.067 (0.195-5.830) | 0.872 | 0.867 (0.154-4.876) |
| Low | 40     |         |              |         |              | 100 |         |              |         |

(B) Same as (A) except VCP expression was used instead of the 13-gene risk score classifier in multivariate analysis.
Table S7 (related to Fig. 5H, I). Univariate and Multivariate Analysis of the RAI-Treated and Entire THCA Cohorts

### A

| Clinical Variable | n       | RAI-treated (n = 226) | THCA (n = 438) |
|-------------------|---------|-----------------------|----------------|
|                   |         | Univariate            | Multivariate   | Univariate            | Multivariate   |
|                   |         | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) |
| Age, years (±SD)  |         |         |              |         |          |         |          |         |          |
| < 50              | 136     | 0.039   | 2.113 (1.039-4.296) | 0.352 | 1.771 (0.532-5.807) | 250     | 0.074   | 1.732 (0.949-3.162) | 0.038   | 1.036 (0.430-2.496) |
| > 50              | 90      |          |              |         |          |         |          |         |          |
| Gender            |         |         |              |         |          |         |          |         |          |
| Male              | 74      | 0.678   | 1.169 (0.560-2.44) | 0.537 | 1.271 (0.593-2.725) | 119     | 0.352   | 1.354 (0.715-2.554) | 0.456   | 1.282 (0.668-2.460) |
| Female            | 152     |          |              |         |          |         |          |         |          |
| Stage             |         |         |              |         |          |         |          |         |          |
| I + II            | 133     | 0.028   | 2.228 (1.068-4.565) | 0.719 | 0.795 (0.227-2.779) | 291     | 0.001   | 2.854 (1.561-5.217) | 0.285   | 1.689 (0.646-4.415) |
| III + IV          | 93      |          |              |         |          |         |          |         |          |
| T stage           |         |         |              |         |          |         |          |         |          |
| T1 + T2           | 116     | 0.010   | 2.758 (1.270-5.99) | 0.053 | 2.274 (0.990-5.223) | 268     | 0.002   | 2.638 (1.421-4.898) | 0.243   | 1.516 (0.754-3.049) |
| T3 + T4           | 110     |          |              |         |          |         |          |         |          |
| Node stage        |         |         |              |         |          |         |          |         |          |
| N0                | 81      | 0.391   | 1.404 (0.646-3.050) | 0.349 | 1.471 (0.650-3.302) | 221     | 0.046   | 1.892 (1.010-3.542) | 0.413   | 1.32 (0.679-2.568)  |
| N1                | 145     |          |              |         |          |         |          |         |          |
| Risk score (13 gene classifier) | |         |         |         |         |         |         |         |         |
| High              | 80      | 8.7x10^-7 | 11.070 (4.248-28.851) | 2.0x10^-6 | 10.577 (3.996-28.001) | 313     | 3.8x10^-7 | 5.523 (2.801-10.038) | 5.0x10^-6 | 4.553 (2.379-8.714) |
| Low               | 146     |          |              |         |          |         |          |         |          |

**Note:** P-values in bold were less than 0.05 and considered statistically significant. Some patients in the RAI-treated (n = 31) and entire THCA cohorts (n = 50) were not included in univariate and multivariate analysis of the THCA dataset due to missing clinical variables.

### B

| Clinical Variable | n       | RAI-treated (n = 226) | THCA (n = 438) |
|-------------------|---------|-----------------------|----------------|
|                   |         | Univariate            | Multivariate   | Univariate            | Multivariate   |
|                   |         | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) |
| VCP expression    |         |         |              |         |          |         |          |         |          |
| High              | 78      | 0.102   | 1.801 (0.890-3.645) | 0.172 | 1.654 (0.804-3.404) | 163     | 0.159   | 1.539 (0.845-2.803) | 0.193   | 1.491 (0.817-2.722) |
| Low               | 148     |          |              |         |          |         |          |         |          |

**Note:** Same as (A) except VCP expression was used instead of the 13-gene risk score classifier in multivariate analysis.1