

**Small-molecule MAPK inhibitors restore radioiodine incorporation in murine thyroid cancers with conditional BRAF activation.**

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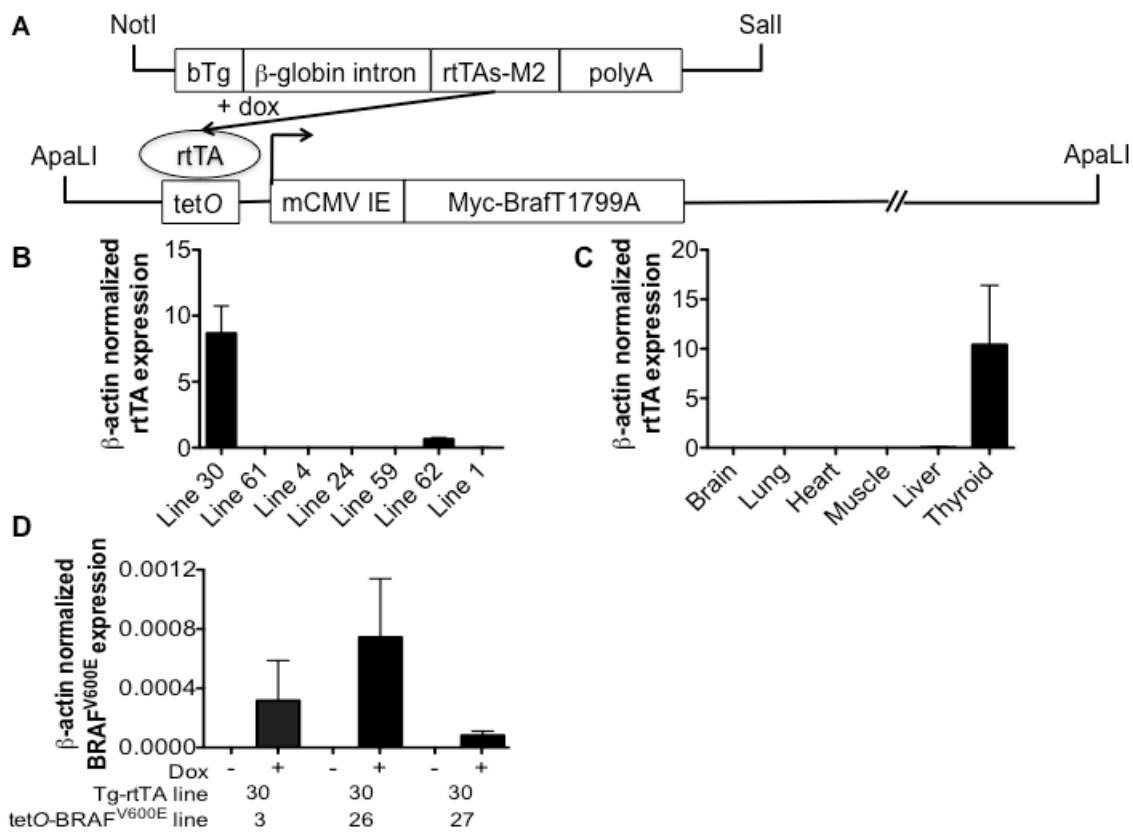
**SUPPLEMENTARY MATERIAL**

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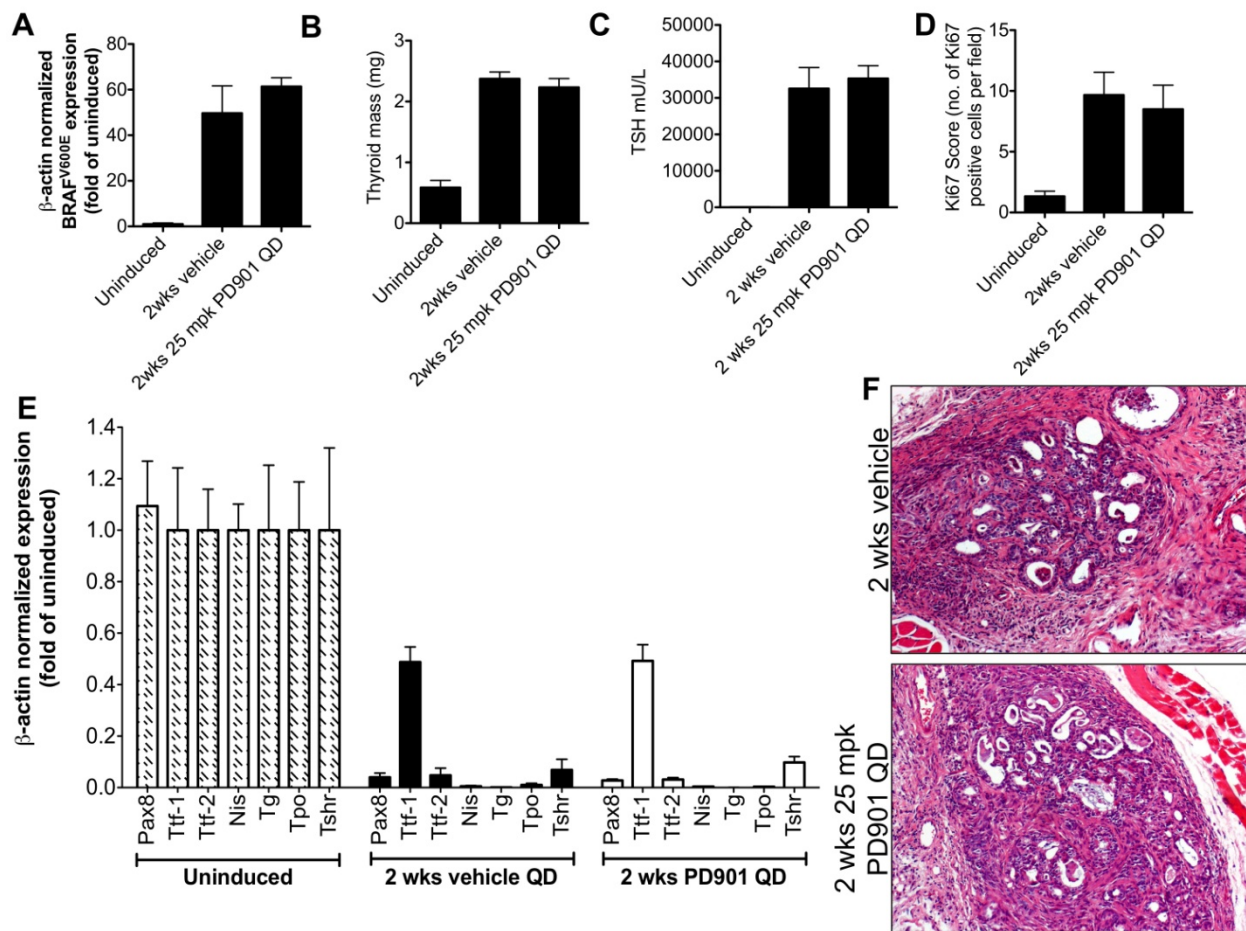
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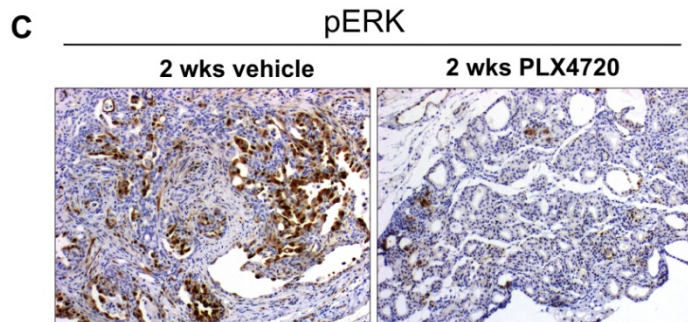
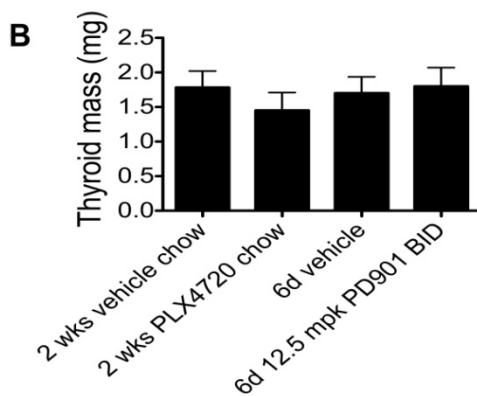
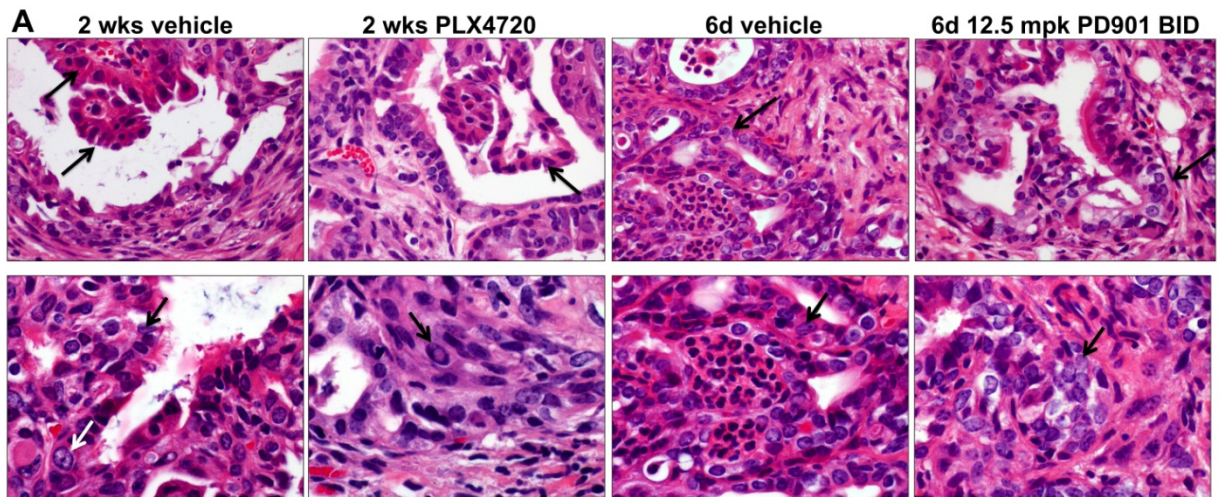
**Figure S1: Mouse model for dox-inducible regulation of BRAF<sup>V600E</sup> expression in thyroid follicular cells.** A) Constructs to generate dox-inducible thyroid-specific expression of BRAF<sup>V600E</sup> in double transgenic mice. *Top:* Reverse tetracycline transactivator cDNA cloned downstream of the bovine thyroglobulin promoter (*Tg-rtTA*). *Bottom:* Myc-tagged Braf<sup>T1799A</sup> cDNA cloned downstream of a multimerized tetO-minimal CMV promoter (*tetO-BRAF<sup>V600E</sup>*). B) Quantitative RT-PCR for rtTA in thyroid tissue of *Tg-rtTA* founder mice from the indicated lines. Data represent rtTA expression normalized to  $\beta$ -actin (n $\geq$ 3). C) Tissue distribution of expression of rtTA in *Tg-rtTA* line 30 (n=2). D) Quantitative RT-PCR of Braf<sup>T1799A</sup> cDNA in thyroid tissues of the indicated line crosses, treated with or without dox for one week (n=2).



**Figure S2:** Two-week treatment of *Tg-rtTA/tetO-BRAF<sup>V600E</sup>* mice with 25 mg/kg PD0325901 once a day shows no restoration of thyroid function: *Tg-rtTA/tetO-BRAF<sup>V600E</sup>* mice were treated with dox for one week and then with 25 mg/kg PD0325901 once a day or vehicle for two weeks in the continued presence of dox. A) Quantitative RT-PCR for mutant BRAF indicates that dox-induced expression levels were not affected by treatment with PD0325901. B) Average thyroid weights of age-matched male *Tg-rtTA/tetO-BRAF<sup>V600E</sup>* mice given 25 mg/kg PD0325901 per day or vehicle control. C) Serum TSH levels of mice treated with or without drug. D) Ki67 index of mice after 2 weeks treatment with drug or vehicle. Data represent manual counts of 3 high power fields per mouse. E) Expression levels of the indicated genes normalized to  $\beta$ -actin as determined by quantitative RT-PCR in the indicated conditions. F) Hematoxylin and eosin staining of histological sections of representative vehicle- or drug-treated mice.



**Figure S3:** Effect of treatment with PLX4720 or PD0325901 12.5 mg/kg twice a day on thyroid weight and histological features. A) H&E-stained sections of representative vehicle- or drug-treated mice. *Top:* 400X. *Left:* Top arrow points to a nuclear groove, lower arrow indicates papillae lined by cells with irregular nuclei. *Middle-left:* Histological features unchanged after 2 wks PLX4720. Arrow points to a papillary structure with atypical nuclei. *Middle-right and Right:* Unchanged histological features after MEK inhibitor treatment. Arrows show irregular clear nuclei of PTC. *Bottom:* 600X. All conditions show similar cellular appearance of PTC cells. *Left:* Black arrow indicates nuclear groove; white arrow shows clear overlapping nuclei. *Middle-left:* Arrow points to nuclear pseudo-inclusion. *Middle-right and Right:* Arrows show irregular clear nuclei of PTC. B) Average thyroid weights of male *Tg-rtTA/tetO-BRAF<sup>V600E</sup>* mice fed with PLX4720 chow or given 12.5 mg/kg PD0325901 twice a day. C) Representative IHC staining for pERK in BRAF-induced thyroid cancers of vehicle or PLX4720-treated mice.



**Table S1****Primers used in this study**

Template	Species	Forward Primer Sequence	Reverse Primer Sequence	Annealing Temperature (°C)
β-actin	Mouse	5'-ctgaaccctaaggccaaccgtg-3'	5'-ggcatacaggacagcacagcc-3'	57
BRAF <sup>V600E</sup>	Human	5'-tcttcatgaagacctcaca-3'	5'-actgtccagtcatcaattca-3'	54
rtTA(s)M2	n/a	5'-caccgcccacagagaaacagta-3'	5'-gtggggcatagaatcggtgtag-3'	60
Pax8	Mouse	5'-cagaaggcgtttgtgacaatga-3'	5'-tgcactttggtccgatgat-3'	60
TTF-1	Mouse	5'-tccagcctatcccatctgaact-3'	5'-caagcgcattcacgtctca-3'	60
TTF-2	Mouse	5'-gcgcatctacaagttcatc-3'	5'-gggatcttgaggagcagtc-3'	57
Tg	Mouse	5'-tgtcccaccaagtgtgaaaa-3'	5'-ccaaggaaagctgttcagc-3'	60
NIS	Mouse	5'-gctcagtctcgctcaaac-3'	5'-cgtgtgacaggccacataac-3'	60
TPO	Mouse	5'-tgacttccaggagcacacag-3'	5'-gcaagttcagtgatgccaga-3'	60
TSHR	Mouse	5'-ctcttaccgagccactg-3'	5'-ttgtcaccggatcttctc-3'	60
DUSP5	Mouse	5'-aggaggagcgtgtctctc-3'	5'-gtggaggcaggatctca-3'	60
PLAT	Mouse	5'-ctggaatagcagtgttctctc-3'	5'-ccctgccttaagacatagcac-3'	55