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Inhibition of Radioiodine Uptake by PBF in Breast Cells is Consistent with Sodium Iodide Symporter Repression in the Thyroid

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Background

Breast cancer is the second most common cancer worldwide and affects 55,000 people in the UK each year. Due to the heterogeneity of the disease, breast cancer can be challenging to treat, particularly for patients with triple negative disease.

Radioiodine treatment has been suggested as a potential treatment for breast cancer patients. Although the sodium iodide symporter (NIS) is not expressed in normal breast tissue, it is expressed in 70 – 80% of breast cancers. However, as in many differentiated thyroid cancers, ¹³¹I uptake in breast cancer is limited due to the low levels of NIS located at the plasma membrane ⁽¹⁾.

Previous studies in thyroid cells have shown that pituitary tumor-transforming gene (PTTG) binding factor (PBF), is capable of altering the subcellular localisation of NIS and sequestering it in cytoplasmic vesicles ⁽²⁾. This interaction can be abrogated by inhibiting the phosphorylation of PBF at tyrosine residue 174 using the Src inhibitor PP1. Mutants of PBF without this key residue are also unable to bind and sequester NIS ⁽³⁾.

With PBF being upregulated in both thyroid and breast cancer ⁽⁴⁾, we hypothesised that the interaction between NIS and PBF may also be apparent in breast cancer and that use of PP1 in breast cancer cells may increase radioiodine uptake.

Aims

- To establish whether upregulation of PBF in breast cancer cells can alter the subcellular localisation and functionality of NIS.
- To determine whether the effect of PBF on NIS can be abrogated using the Src inhibitor, PP1, as demonstrated in thyroid cells.

Conclusions

Taken together these data support the hypothesis that PBF can interact with and alter the subcellular localisation of NIS within breast cancer cells. PBF also decreased radioiodine uptake in breast cancer cells in a similar manner to that demonstrated in thyroid cancer. *In vitro* radioiodine uptake experiments have shown that radioiodine uptake can be restored using the Src inhibitor, PP1, which prevents phosphorylation of PBF at residue Y174. This suggests that PP1 may have therapeutic benefits and potentially increase uptake of radioiodine in breast cancer patients.

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PBF reduces ¹²⁵I uptake in breast cancer cells

Uptake of ¹²⁵I was assessed in MCF-7 and MDA-MB-231 cells transiently transfected with NIS and PBF. MCF-7 cells demonstrated increased radioiodine uptake when transfected with NIS. This uptake was significantly decreased with PBF co-transfection (25% reduction, $p=0.011$) (Fig. 1A). Similar reduction was also observed in MDA-MB-231 cells (30% reduction, $p=0.002$) (Fig. 1A). As demonstrated previously all trans retinoic acid (ATRA) and dexamethasone increased NIS expression and radioiodine uptake in MCF-7 cells ⁽⁵⁾ (Fig. 1C). This effect was significantly reduced when ATRA/dexamethasone treated cells were also transfected with PBF (22% reduction, $p=0.03$) (Fig. 1C).

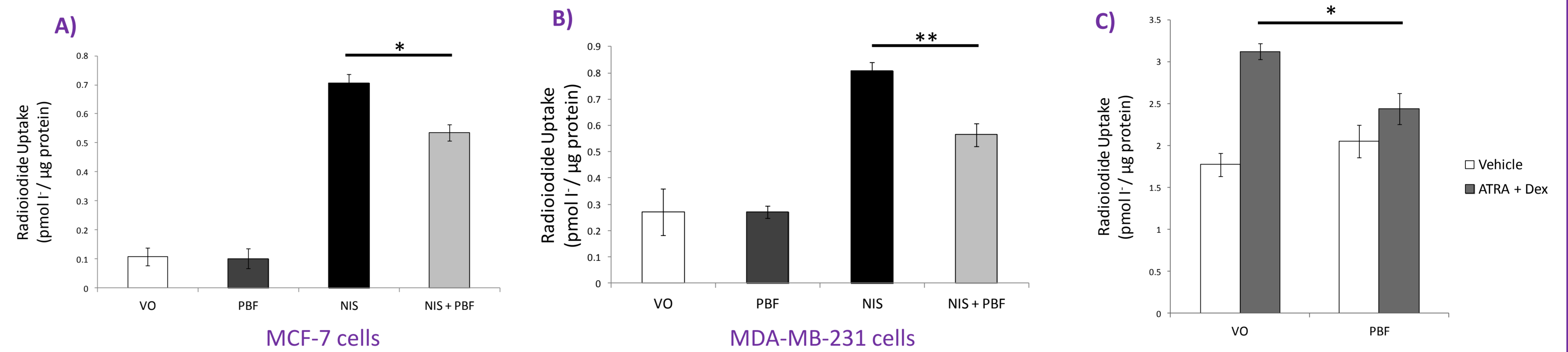
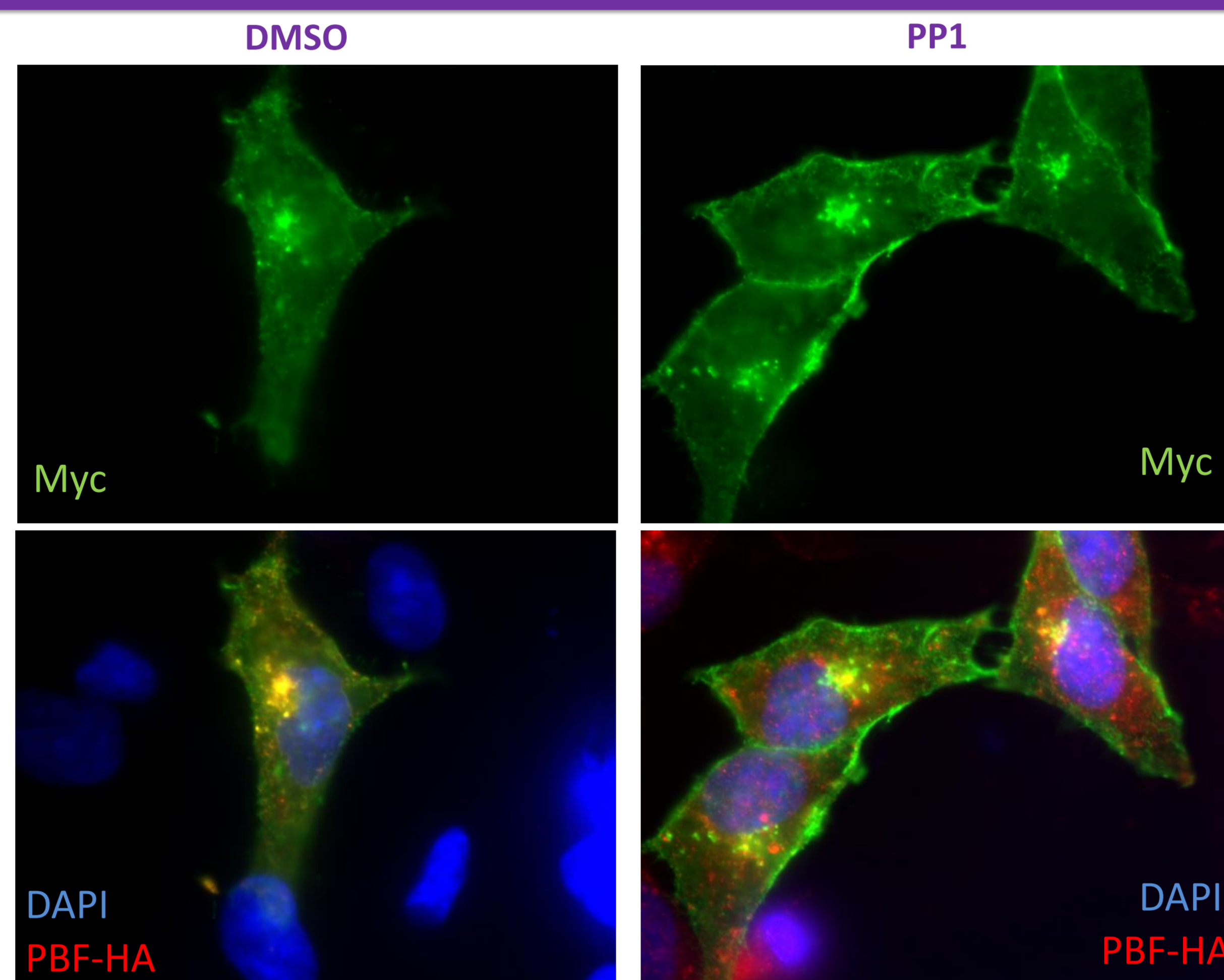


Figure 1. Iodine uptake studies were conducted in MCF-7 (A) and MDA-MB-231 (B) cells transfected with vector only (VO), PBF and NIS cDNA, $n=3$ (C) MCF-7 cells were treated with vehicle (ethanol and DMSO) or 10^{-6} M ATRA and 10^{-7} M Dexamethasone and transfected with either VO or PBF, $n=3$. * = $p<0.05$, ** = $p<0.01$

Inhibition of PBF phosphorylation increases plasma membrane NIS



MDA-MB-231 cells transfected with NIS-MYC (green) and PBF-HA (red) were treated with the Src inhibitor PP1, and localisation of the two proteins visualised (Fig. 2). Cells treated with the vehicle control DMSO displayed co-localisation (yellow) of PBF and NIS in intracellular vesicles with some plasma membrane NIS evident.

Cells treated with PP1 displayed less colocalisation between NIS and PBF, with a noticeable increase in NIS at the plasma membrane.

Figure 2. NIS localisation in MCF-7 cells was assessed using immunofluorescence studies of cells transiently transfected with MYC-tagged NIS and PBF-HA cDNA which were probed using rabbit anti-HA and mouse anti-MYC antibodies. Cells were imaged using Zeiss Axioplan fluorescent microscope.

Inhibition of PBF phosphorylation restores ¹²⁵I uptake

MCF-7 cells transfected with NIS and PBF were treated with the Src inhibitor PP1, and their ability to uptake iodine assessed. In vehicle only treated cells there was a reduction in radioiodine uptake between NIS and NIS + PBF transfected cells (25% reduction, $p=0.008$) (Fig. 3A). Cells transfected with NIS + PBF and treated with PP1 displayed increased uptake compared to control treated cells (24% increase, $p=0.011$) (Fig. 3A). This was also observed in MDA-MB-231 cells with an initial reduction of 33% with PBF co-transfection ($p=0.003$) which was restored after treatment with PP1 ($p=0.0008$) (Fig. 3B). In ATRA/dexamethasone treated cells, PBF transfection reduced radioiodine by 22% ($p=0.03$), and PP1 treatment increased radioiodine uptake by 43% ($p=0.03$) (Fig. 3C).

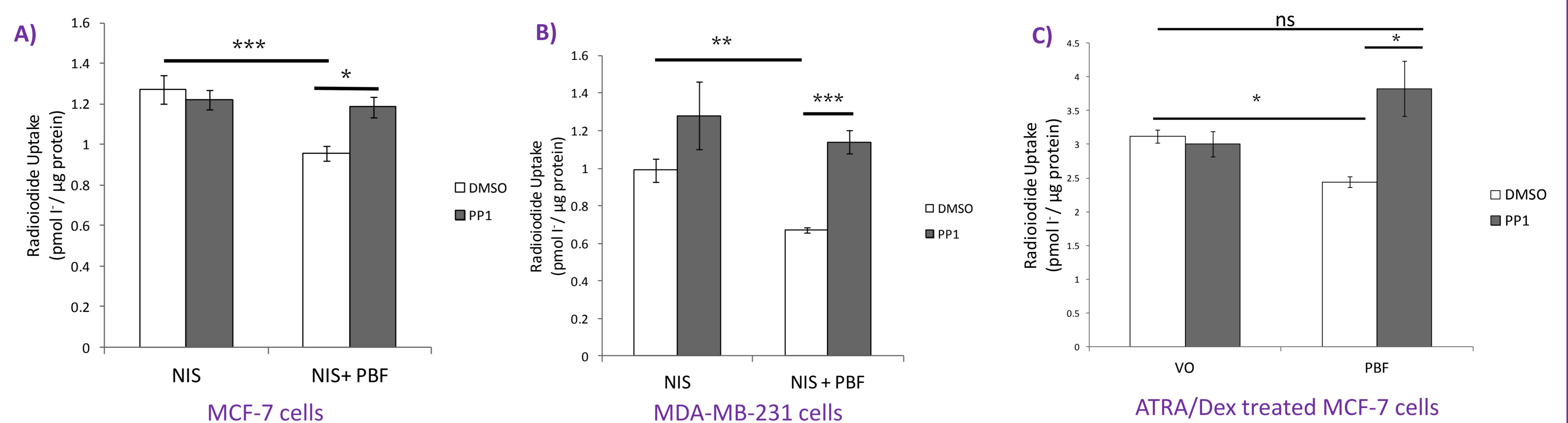


Figure 3. Radioiodine uptake was assessed in MCF-7 (A) and MDA-MB-231 (B) cells transfected with NIS and NIS + PBF that had been treated with DMSO or 2μM PP1. $n=3$ (C) Radioiodine uptake was assessed in MCF-7 cells treated with 10^{-6} M ATRA and 10^{-7} M Dexamethasone and transfected with either VO or PBF and treated with DMSO or 2μM PP1. $n=3$. ns = not significant * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

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