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# Ionic modulation of immune checkpoint proteins

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## Abstract

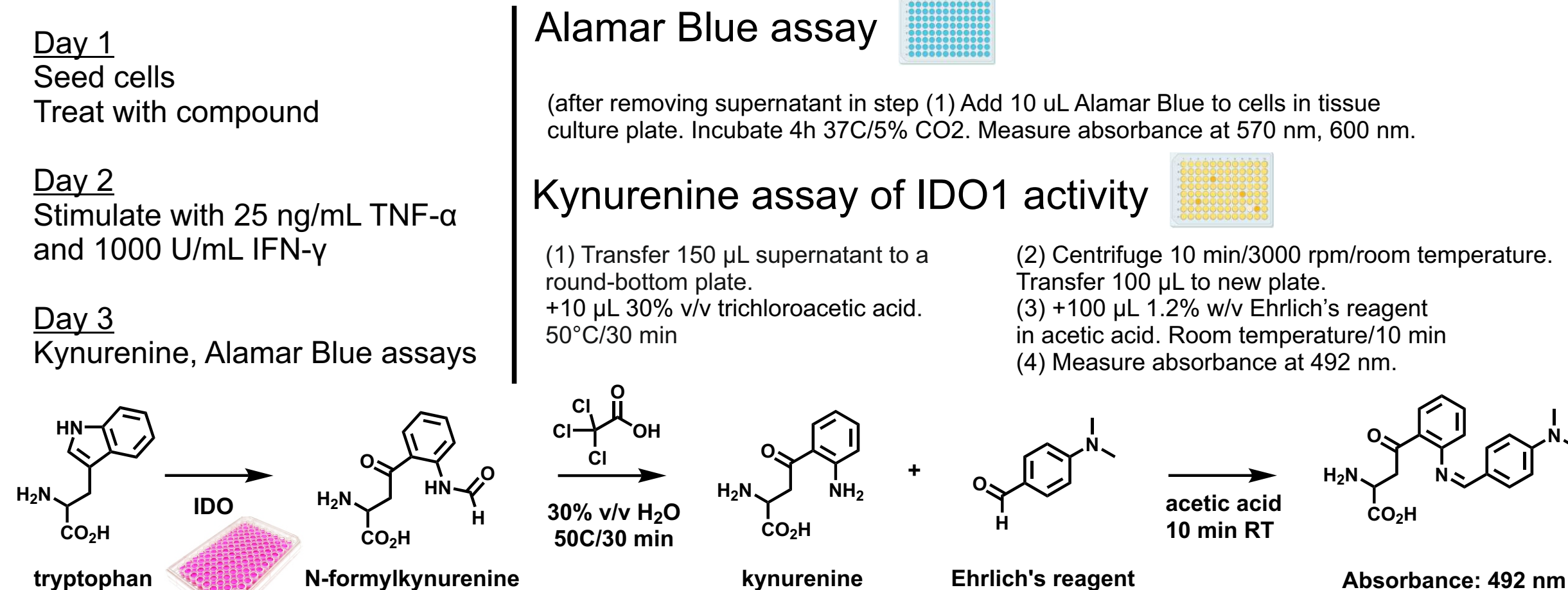
Despite extensive basic and clinical research on immune checkpoint regulatory pathways, little is known about the effects of the ionic tumour microenvironment on immune checkpoint expression and function.

We screened effects of ion channel modulating compounds on IDO1 activity. Here, we describe a mechanistic link between  $\text{Na}^+/\text{K}^+$  ATPase inhibition by cardiac glycosides and activity of indoleamine-2',3'-dioxygenase (IDO1), a well-characterized immune checkpoint.

IDO1 catalyses the rate-limiting step of tryptophan catabolism and inhibits the immune response to the tumour by local depletion of tryptophan, an amino acid essential for anabolic functions in cancer and T cells.

## Methods

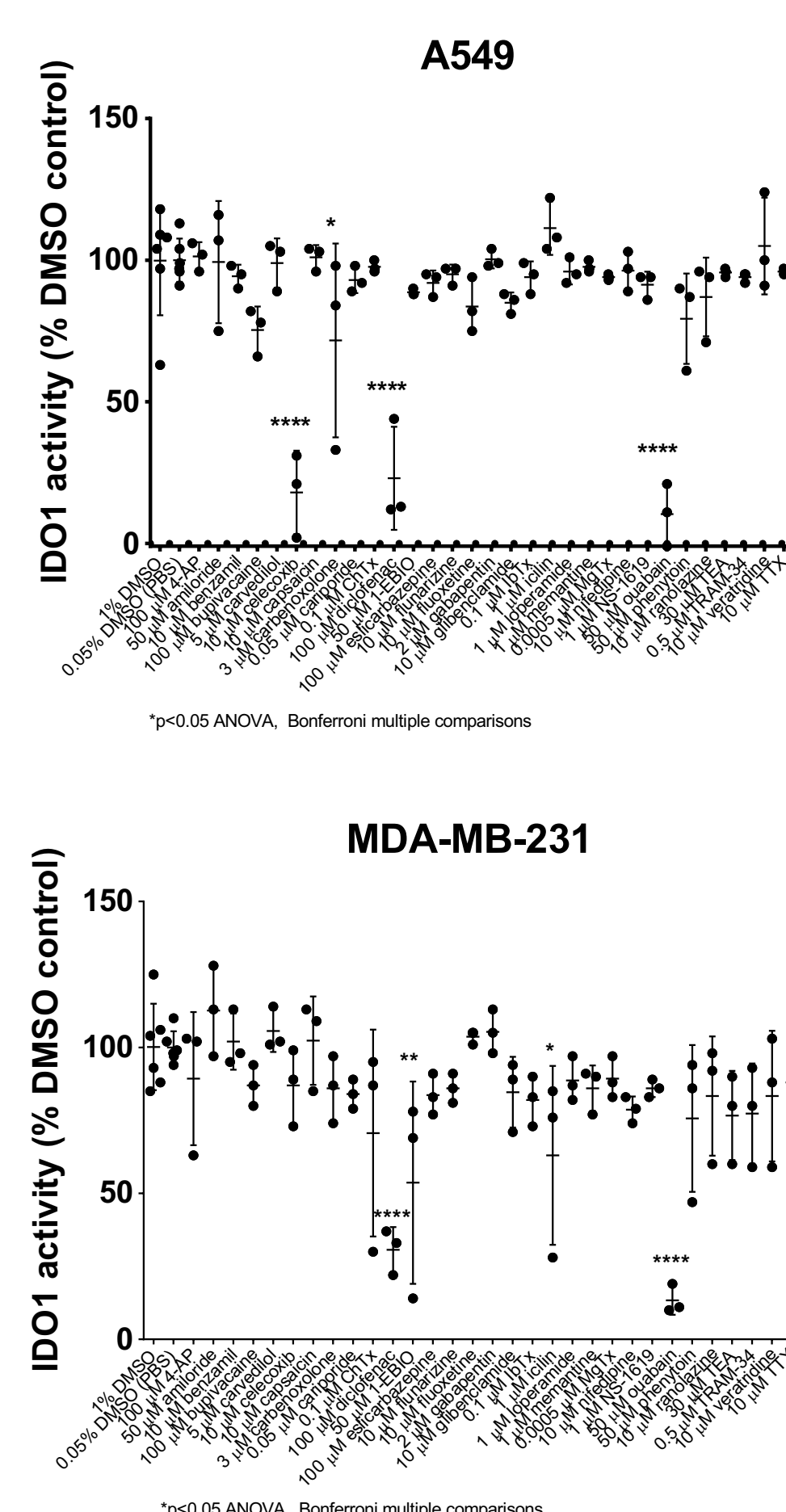
### IDO1 activity is measured using a kynurenine assay.



### Ion channel drug screen of IDO1 activity

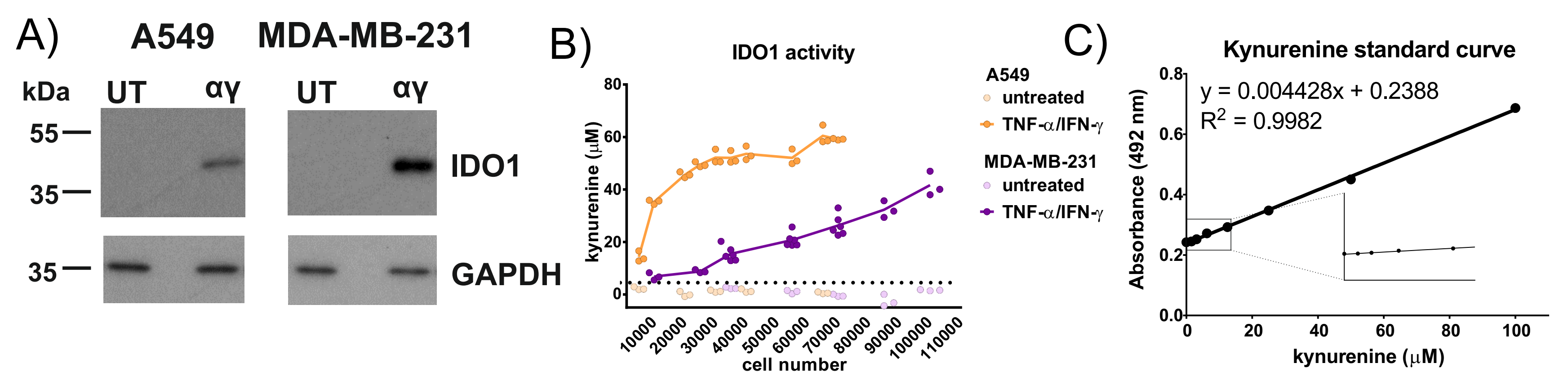
Drug/toxin	Target(s)	Effect
4-aminopyridine	K <sup>+</sup> channels	Inhibitor
Amiloride	ASIC channel	Inhibitor
Benzamil	Na <sup>+</sup> /Ca <sup>2+</sup> exchanger, ENaC, TRPP3, TRPA1 channels	Inhibitor
Bupivacaine	Na <sup>+</sup> K <sup>+</sup> channels	Inhibitor
Caneledil	K <sub>v</sub> 1.5, K <sub>v</sub> 2.3 K <sup>+</sup> channels, adrenoceptors	Inhibitor
Celecoxib	L-type Ca <sup>2+</sup> and K <sub>v</sub> 7 channels	both
Diclofenac (sodium)	K <sub>v</sub> 7 channels	Activator
Etacalcabazine acetate	VGCC	Inhibitor
Flunarizine dihydrochloride	L-type and T-type Ca <sup>2+</sup> channels	Inhibitor
Fluxetidine hydrochloride	K <sub>v</sub> 4.3 channels	Inhibitor
Iberiotoxin	K <sub>v</sub> 3.1 K <sup>+</sup> channels	Inhibitor
Memantine hydrochloride	NMDA receptor	Inhibitor
Nifedipine	L-type Ca <sup>2+</sup> channels	Inhibitor
NS-1619	K <sub>v</sub> 2.1 K <sup>+</sup> channels	Activator
Ouabain	Na <sup>+</sup> /K <sup>+</sup> ATPase	Inhibitor
Phenylethanol	VGCC, HERG	Inhibitor
Tetraethylammonium chloride	K <sup>+</sup> channels - all	Inhibitor
Tetrodotoxin	VGCC	Inhibitor
TRAM-34	K <sub>v</sub> 3.1 K <sup>+</sup> channels	Inhibitor
Veratridine	VGCC	Activator
1-EBIO	K <sub>v</sub> 3.1, K <sub>v</sub> 2 channels	Activator
Capsaicin	TRPV1 channels	Inhibitor
Carbenoxolone	Pannexin-1 (Gap junction channel)	Inhibitor
Cariporide	NHE1	Inhibitor
Charybotoxin	K <sub>v</sub> 1.1, K <sub>v</sub> 1.2, K <sub>v</sub> 1.3 K <sup>+</sup> channels	Inhibitor
Cisapride	VGCC, $\alpha$ 1A-2, delta	Inhibitor
Glibenclamide	TRPA1, KATP channels	Inhibitor
Icilin	TRPM8 channel	Activator
Loperamide hydrochloride	type, T-type Ca <sup>2+</sup> , HCN channels, NMDA, $\mu$ -opioid receptor	Inhibitor
Margatoxin	K <sub>v</sub> 1.3, K <sub>v</sub> 1.6	Inhibitor
Ranolazine	VGCC	Inhibitor

A library of 31 compounds targeting ion channels or pumps was screened in the kynurenine assay measuring IDO1 activity in TNF- $\alpha$ /IFN- $\gamma$  stimulated lung cancer (A549) and breast cancer (MDA-MB-231) cells. Points (right panel) represent technical replicates and data are normalized to their respective DMSO control, 0.05% or 1% DMSO in PBS.

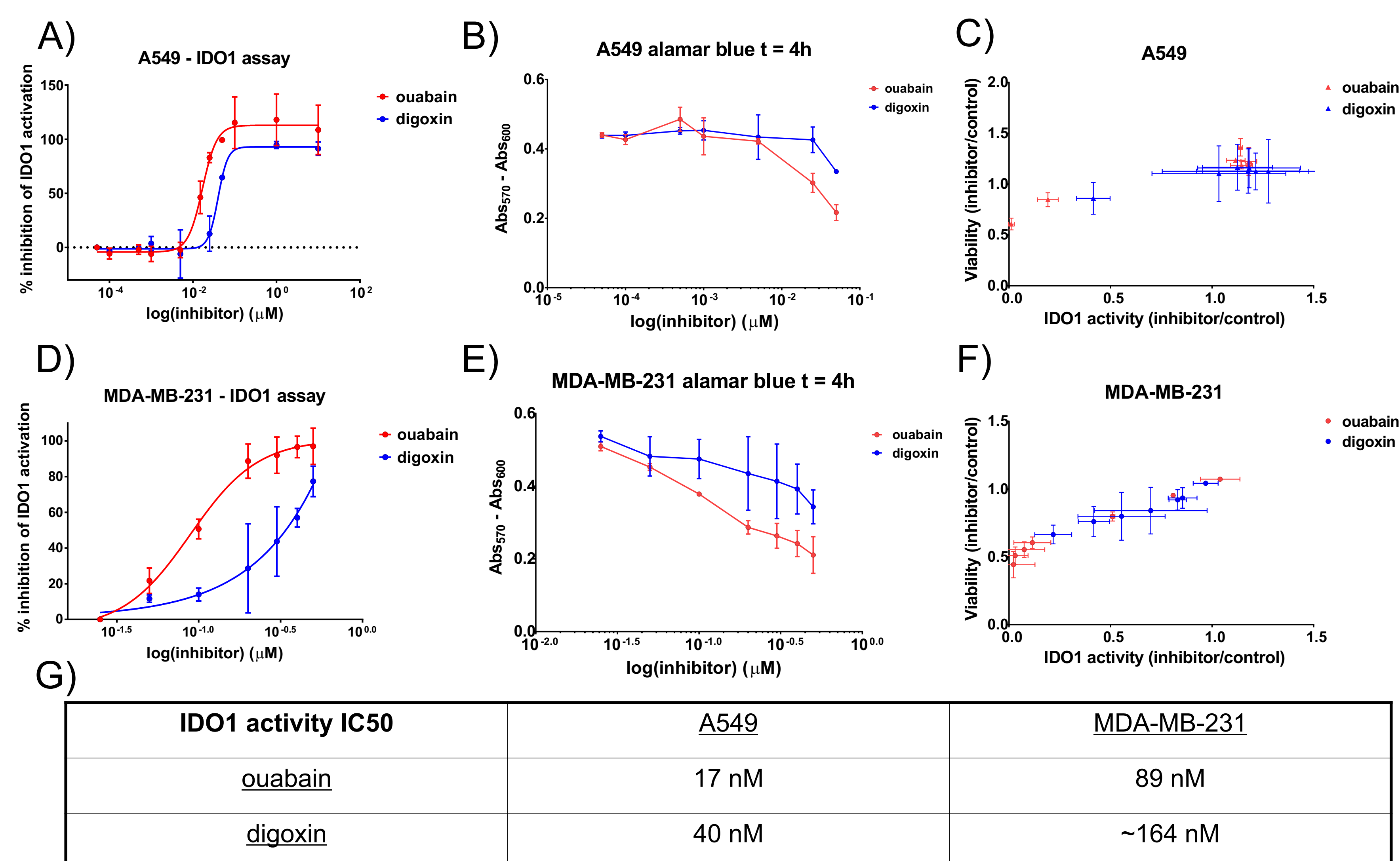


## Results

### TNF- $\alpha$ /IFN- $\gamma$ stimulates IDO1 expression in lung cancer A549 and breast cancer MDA-MB-231 cells.

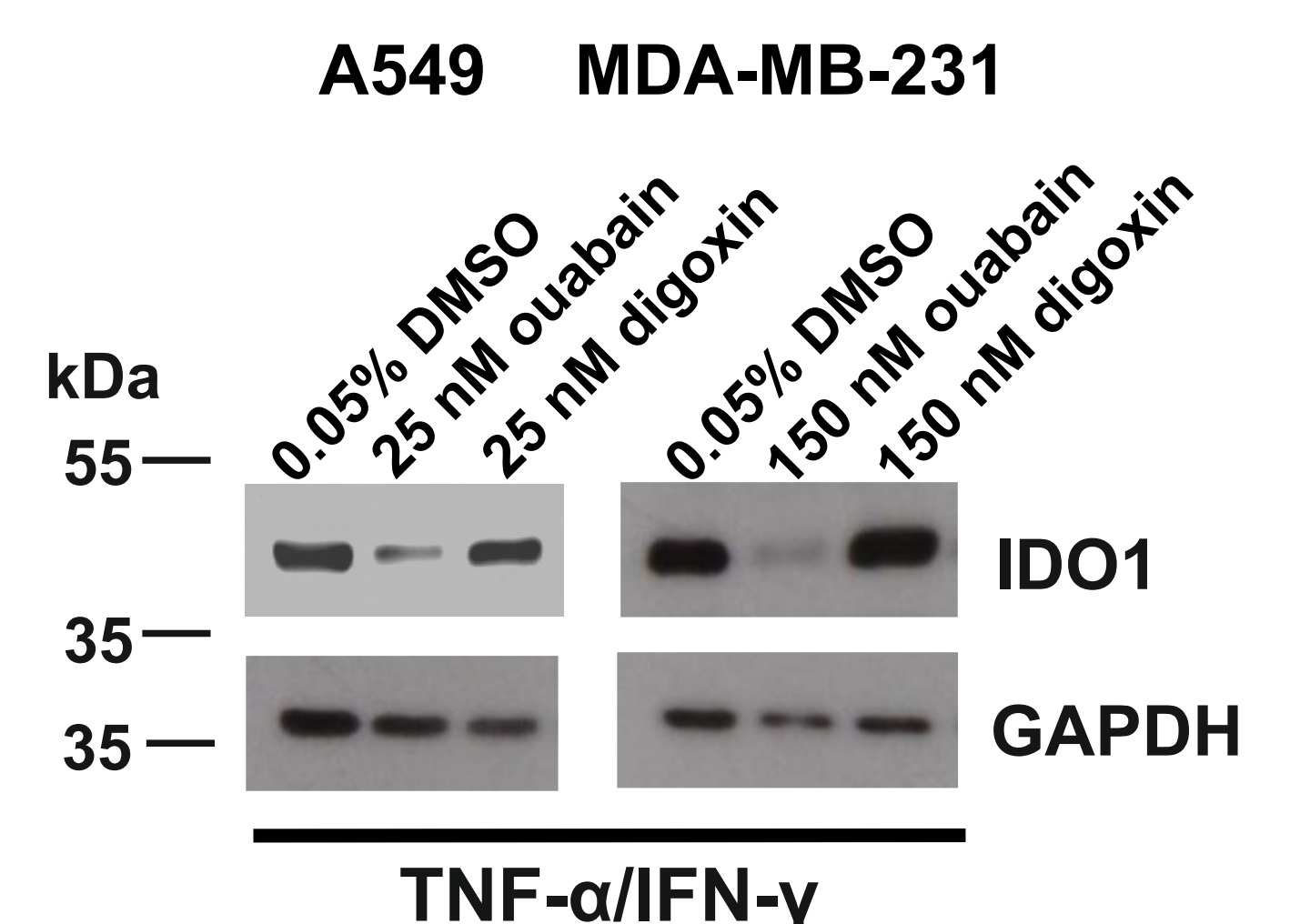


### Cardiac glycosides ouabain and digoxin inhibit kynurenine production in A549 and MDA-MB-231 cancer cells with a modest impact on cell survival.



A) Dose-response curves for ouabain and digoxin treatment of TNF- $\alpha$ /IFN- $\gamma$  stimulated A549 cells. B) Alamar Blue survival assay after 4h incubation with reagent. C) Comparison of IDO1 activity vs. viability as measured by Alamar Blue in A549 cells. D) Dose-response curves for ouabain and digoxin treatment of TNF- $\alpha$ /IFN- $\gamma$  stimulated MDA-MB-231 cells. E) Alamar Blue survival assay after 4h incubation with reagent. F) Comparison of IDO1 activity vs. viability as measured by Alamar Blue in MDA-MB-231 cells. G) Calculated IDO1 activity IC50s from fitting the dose-response curves by non-linear least squares regression.

### Ouabain, but not digoxin, downregulates IDO1 expression in A549 and MDA-MB-231 cancer cells.



Western blot of IDO1 expression in TNF- $\alpha$ /IFN- $\gamma$  stimulated cells treated with cardiac glycosides. A549 cells were treated with 0.05% DMSO, 25 nM ouabain, or 25 nM digoxin (left panel). MDA-MB-231 cells were treated with 0.05% DMSO, 150 nM ouabain, or 150 nM digoxin (right panel).

## Cardiac glycosides decrease activity of immune checkpoint protein IDO1 in cancer cells.