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Allosteric non-competitive small molecule selective inhibitors of CD45 tyrosine phosphatase suppress T-cell receptor signals and inflammation in vivo

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Supplemental Figures and Tables

Docking of first generation compounds onto CD45

The "hits" **28p** or **37p** were docked at a pocket of CD45 formed by the interface of D1-D2 and the linker that joins these domains. Residues from D1 and D2 form hydrogen bonds and hydrophobic contacts with compounds **28p** or **37p**. For both inhibitors, the D2 domain contributes the majority of predicted contacts (Figure S-1).

Docking predicts that the sulfonic acid groups of **28p** form hydrogen bonds with the D2 domain backbone carbonyl of Tyr957 and Phe997, as well as Lys1136 and Lys 1181 side-chain amino groups, and the Ser1163 side-chain hydroxyl group. A hydroxyl moiety of **28p** contacts the D1 domain at the Asp766 side chain via hydrogen bonding. One central phenyl group in the inhibitor is sandwiched between the methylene groups of Glu801 of the D1 domain and Met999 of the D2 domain. Tyr1001 in D2 packs against a naphthyl group.

As for **37p**, docking predicts that its carbonyl groups make tight hydrogen bonds with the D2 domain amino groups of Lys1003 and Asn1159. The phenyl ring of the compound is sandwiched between Leu805 of the D1 domain and the side chain methylene groups of Gln1174 of the D2 domain. One of the naphthoquinone rings of **37p** packs against a hydrophobic wall composed of the side chains of Met999, Phe997 and Val1006 in the D2 domain.

Figure S-1. Docking of compounds at a pocket region near the CD45 D1 D2 domain interface.

(**A**) 2D structures of hit compounds **28p** and **37p**

(**B, C**) **28p** and **37p** docked at the D1-D2-linker interface of CD45, the putative allosteric binding site. D1 domain is shown in green, and D2 domain in yellow. Panel B shows the detailed interactions with the binding pocket. Panel C shows the binding pocket as a solvent-excluded surface. Hydrogen bond interactions are shown as dashed lines.

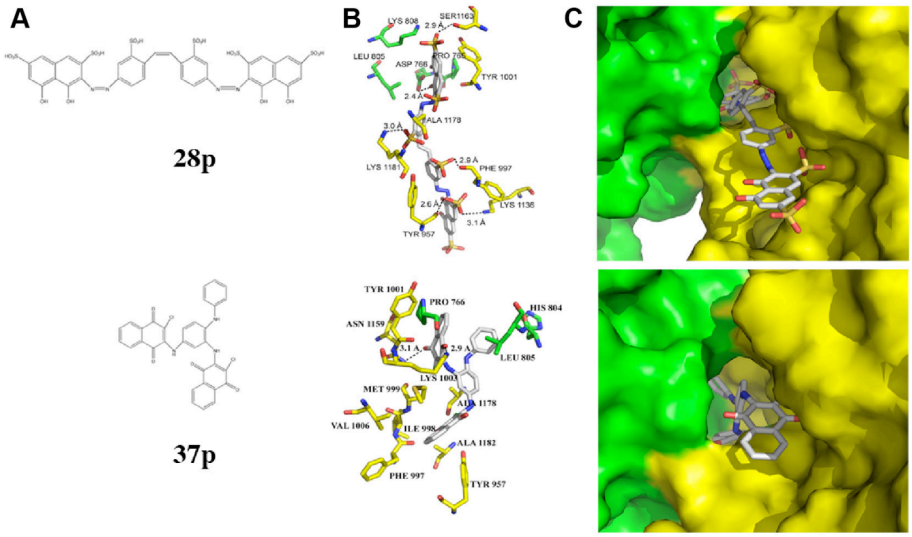
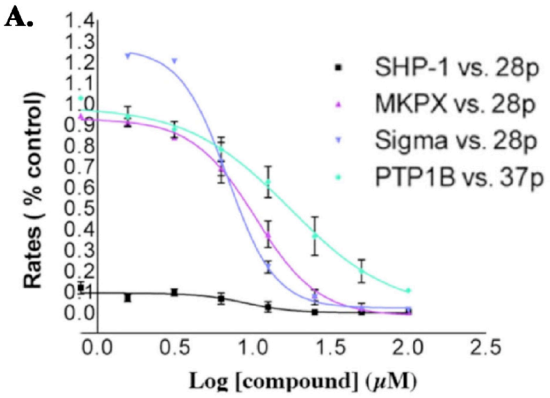
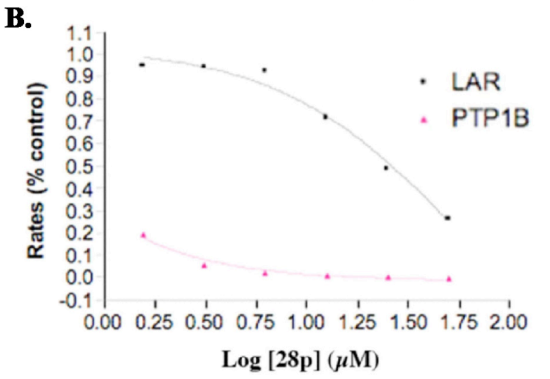


Figure S-2. Specificity counter-assays for different PTPs and different substrates

(**A**) Protein tyrosine phosphatases SHP-1, MKPX, and PTP-Sigma were incubated with **28p**, and PTP1B was tested against **37p**, using a generic pNPP substrate. The curves are generated from activity rates at different concentrations of inhibitor. Data are shown as a percent of untreated control. (**B**) The inhibition by **28p** on LAR and PTP1B are shown. (**C**) DIFMUP substrate was used on CD45 and PTPN22, and assayed for fluorescence as a readout of phosphatase activity after 20 minutes incubation with the indicated concentrations of **211**. Data are shown as a percent of control untreated enzyme.





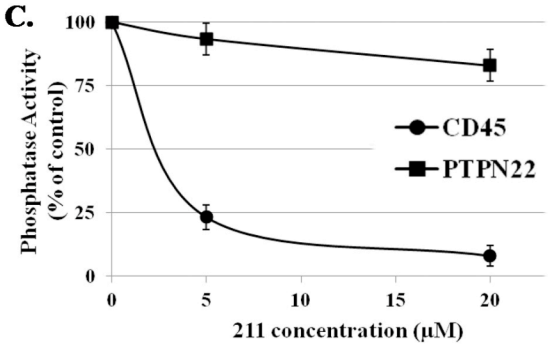


Figure S-3. Quantification of wild type and mutant CD45 phosphatase activity

The basal phosphatase activity of wild type and mutant CD45 was assayed in a modified Malachite Green assay. Free phosphate liberated from a phospho-peptide substrate was quantified by measurement of OD_{620}. All CD45 proteins show similar phosphatase enzymatic activity, equivalent to wild type. This assay controls for intact enzymatic activity of the mutants and aids in the interpretation of the inability of **211** to inhibit the mutant proteins.

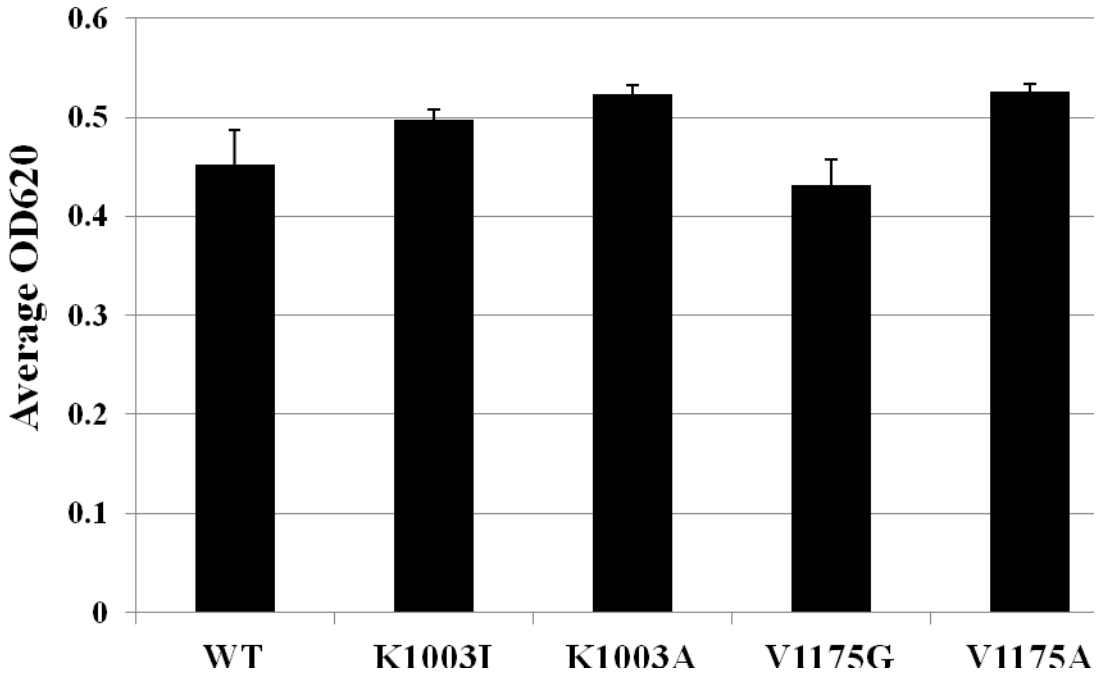


Figure S-4. Inhibitors alter the conformation of CD45

Far-UV range circular dichroism spectra of CD45 D1-D2 in 10 mM Tris-H_3PO_4 buffer, pH 7.0. Measurements were made in a 0.5 mm cuvette

(**A**) 0.04 mg/mL CD45 alone, or CD45 co-incubated for 30 minutes with 0.01 mg/mL compound **211**, or 0.01 mg/mL compound **57p** which is an inactive compound used as control. Heat-denatured CD45 is also shown as control. Removal of compound **211** (by centrifugation) did not result in the renaturation of CD45 (data not shown) after 30 min.

(**B**) 0.04 mg/mL LAR alone, or LAR co-incubated for 30 minutes with 0.01 mg/mL compound **211**. Compound **211** alone is shown as control.

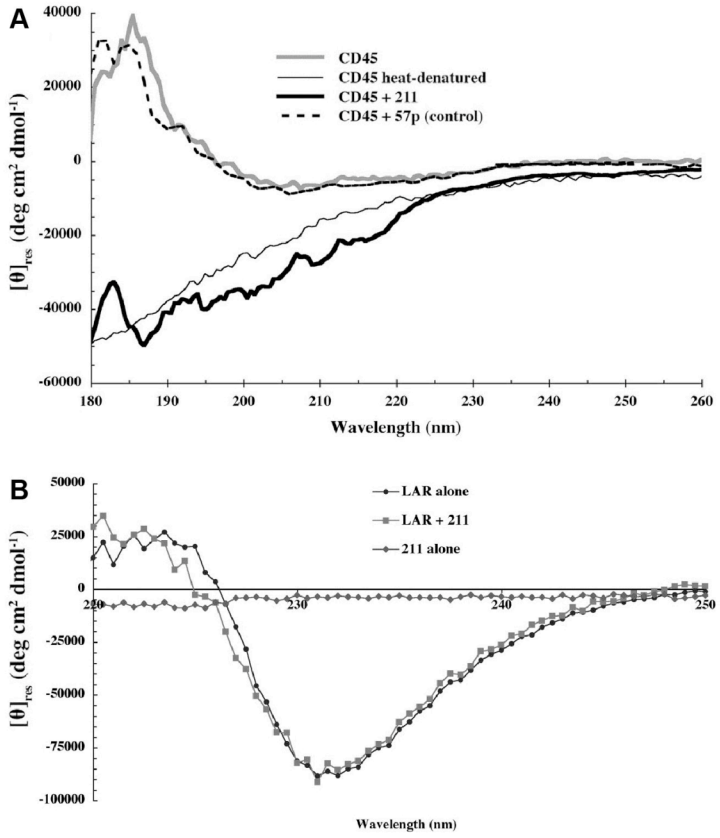


Figure S-5. : **Effect of 211 treatment on Lck phosphorylation in unstimulated cells**
EL4 cells were treated with vehicle or 0.5 μM **211** for 24 hours ± stimulation of the TCR with plate-bound anti-CD3 antibody. Cells were lysed and samples were blotted for p-Lck Y393 as a measure of T cell signaling activation. **211** inhibited phosphorylation in activated splenocytes, but had no effect on p-Lck Y393 levels in unstimulated cells. Actin protein is shown as a loading control.

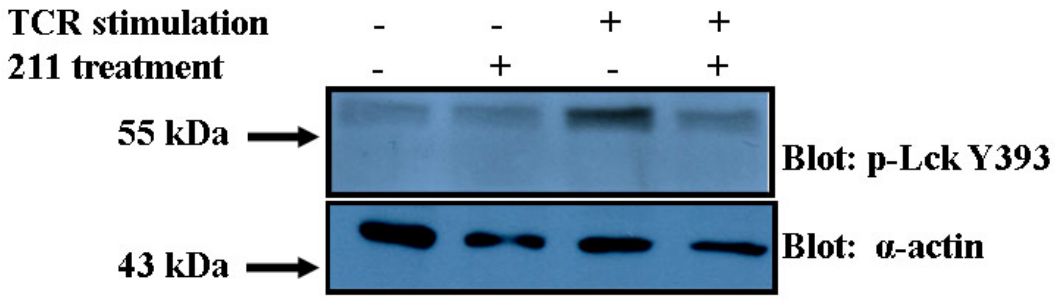


Figure S-6. Histological staining of sections obtained from mouse foot-pad.

Tissue sections were stained with H&E and viewed under 10x magnification. Cell infiltration is apparent in the right footpad of antigen challenged group A, but it is diminished in antigen challenged drug-treated groups B and C, and absent in the unchallenged left footpad.

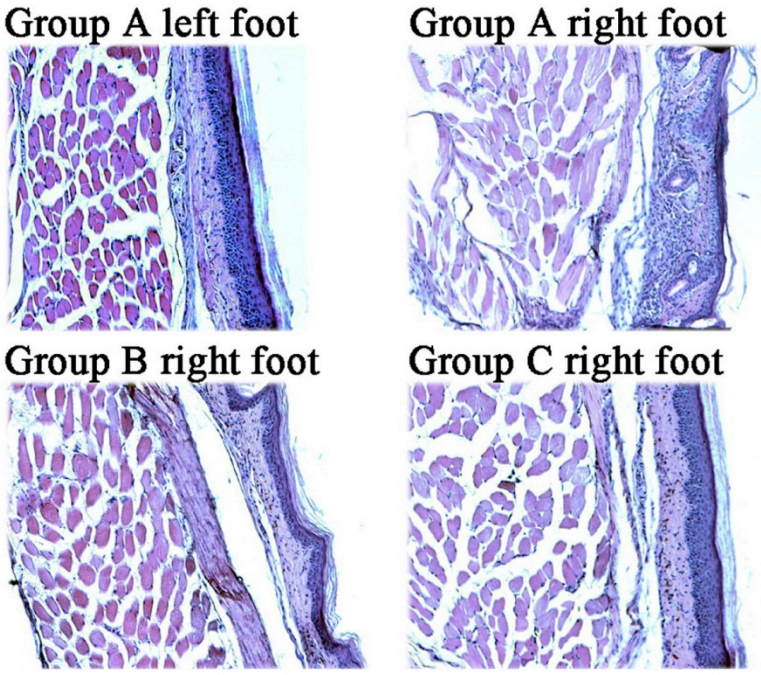


Table S-1. Cell viability with 0.5 μM CD45 inhibitor treatment for 24 hours

	Cell viability (MTT assay, % of untreated control) ± SEM			
Compound	**Jurkat**	**J45.01**	**KB**	**HEK 293**
37p	98 ± 0.6	95 ± 0.7	94 ± 4.5	98 ± 2.0
210	95 ± 1.4	94 ± 1.0	94 ± 0.5	110 ± 3.8
211	82 ± 5.1	91 ± 3.0	94 ± 3.9	103 ± 3.1
214	101 ± 3.2	98 ± 1.1	99 ± 4.0	102 ± 1.1
215	83 ± 3.1	88 ± 3.1	98 ± 3.5	110 ± 5.2
216	89 ± 1.5	89 ± 2.0	95 ± 1.6	100 ± 3.8

Table S-2. Additional 211 selectivity controls. The inhibitory capacity of compound **211** was tested against additional PTPases related to CD45, using pNPP substrate. The proteins had PTP enzymatic activity, but **211** at the highest concentration tested (40 or 50 μM) did not significantly inhibit these enzymes.

	IC_{50} (μM)				
Compound	**PTPN22**	**PEP**	**PEST**	**PRL-2**	**TC-PTP**
211	>40	>40	>40	>50	>50