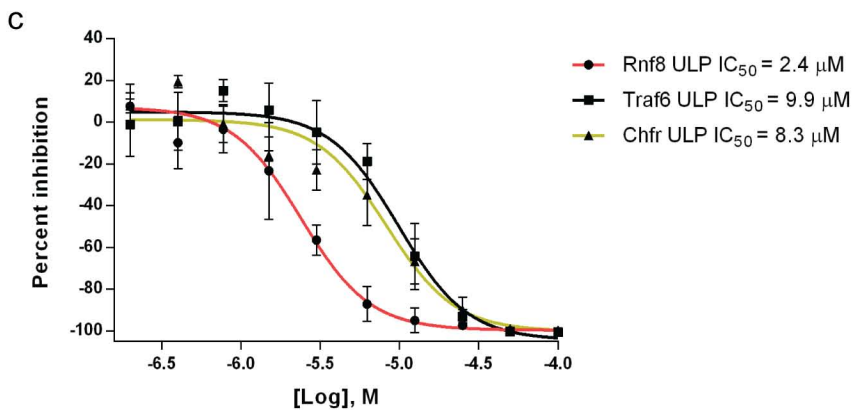
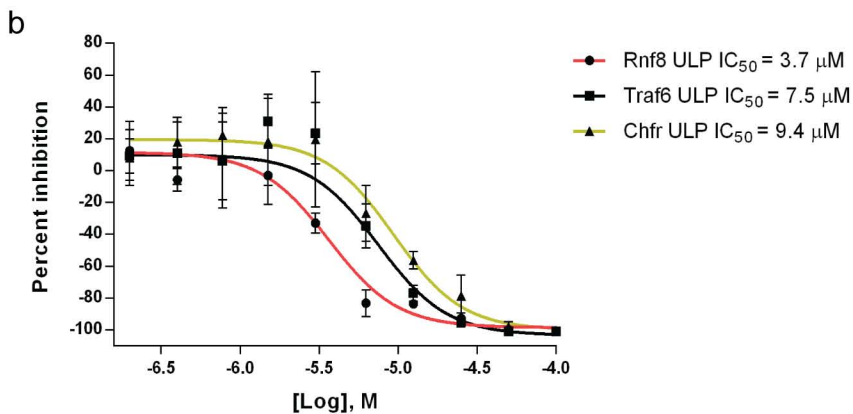
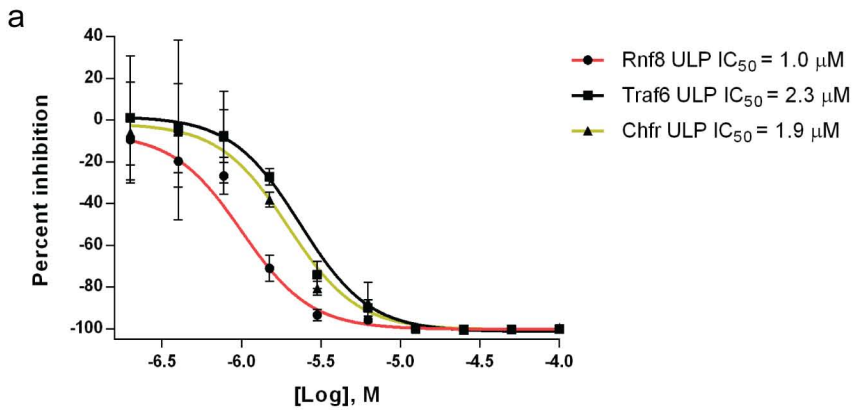


A Generic Platform for Cellular Screening Against Ubiquitin Ligases

Authors

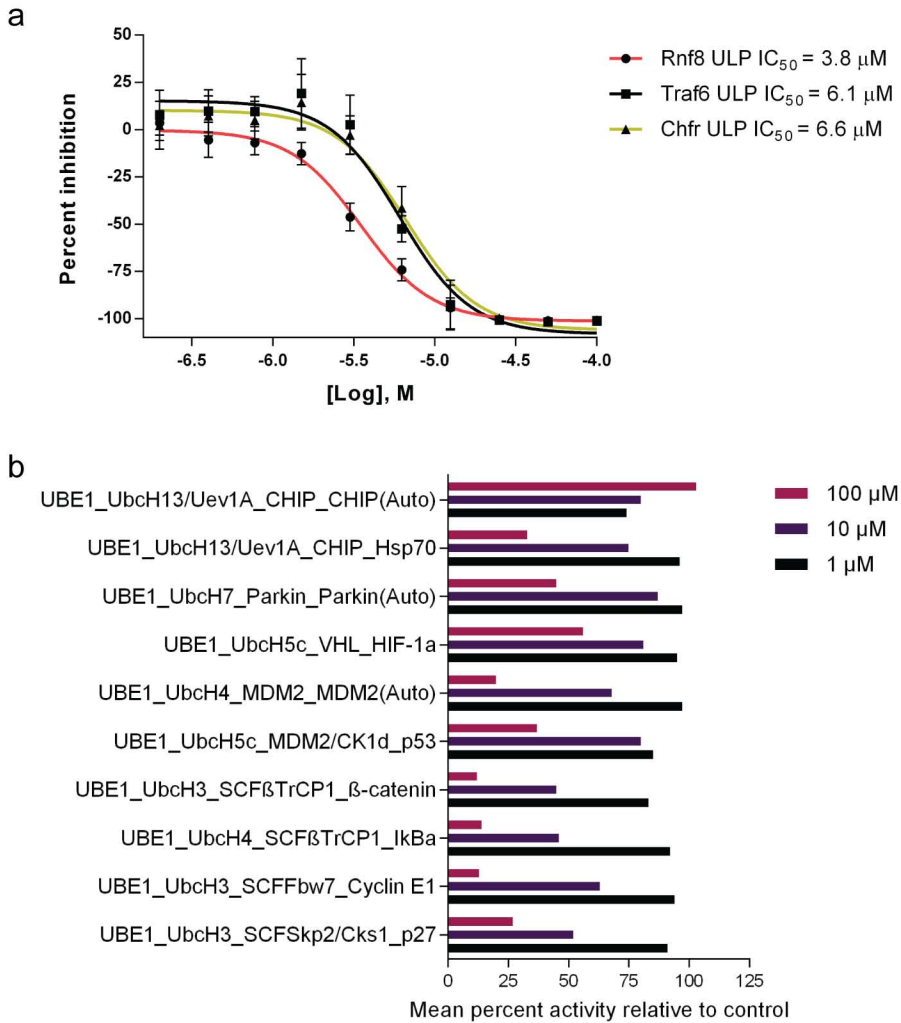
Timurs Maculins, Nikki Carter, Thierry Dorval, Kevin Hudson, J. Willem M. Nissink, Ronald T. Hay and Husam Alwan.

Supplementary Figure S1



Supplementary Figure S1. Examples of compounds with different selectivity profiles. Dose-response curves for (a) a deprioritized compound lacking preferential inhibition of the Rnf8 ULP assay, (b) a compound exhibiting selectivity for Rnf8 ULP assay less than 0.5 log units and (c) a compound exceeding our minimal selectivity threshold of 0.5 log units against Rnf8 ULP assay (n=5).

Supplementary Figure S2



Supplementary Figure S2. Non-specific effect of compound-2. **(a)** Compound lacking preferential inhibition of Rnf8 in the ULP assays (n=5). **(b)** Compound-2 exhibits non-selective inhibition of the majority of indicated ubiquitylation cascades *in vitro* (n=2).

Supplementary Table S1. Details of PCR primers used in this study.

Primer	Description	Primer sequence
9	Sgfl RNF8 5'	GACCGCGATCGCCATGGGGGAGCCCGGCTTCTT
10	Pmel RNF8 3'	GTCGGTTTAAACTCAGAACAATCTCTTTGCTTTTCGTTCCC
25	Sgfl 3XUIM-7A 5'	CACCGCGATCGCCATGACAGAGGAGGAACAGTTCGCCCTTG
26	Pmel 3XUIM-7A 3'	CTTGGTTTAAACCTAGGAAGGCCTACAGGAATTCAGGGATTCTGC
37	RNF8 t1207a FW	TGTGCTAGAGAATGAGCTCCAAAGTATTATTTGTTTCAGAATACTTC
38	RNF8 t1207a RV	GAAGTATTCTGAACAAATAATACTTTGGAGCTCATTCTCTAGCACA
62	Sgfl-CHFR 5'	CACGGCGATCGCCATGGAGCGGCCCGAGGAAGGC
63	Pmel-CHFR 3'	TTCTGTTTAAACTTAGTTTTTGAACCTTGTCTGTTACAGAT
121	Sgfl-TRAF6 5'	TTGTGCGATCGCCATGAGTCTGCTAAACTGTGAA
122	Pmel-TRAF6 3'	GGGGGTTTAAACTCATACCCCTGCATCAGTACTT
172	TRAF6 R88A FW	ACGCCATGCGGCCATGCGTTCTGCAAAGCCTG
173	TRAF6 R88A RV	CAGGCTTTCAGAACGCATGGCCGCATGGCGT
182	FW CHFR W240A	CTTGCTACTCGGGCGCGATGGAGCGCTCGT
183	RV CHFR W240A	ACGAGCGCTCCATCGCGCCCGAGTAGCAAG

Supplementary Table S2. Details of constructs used in this study.

Construct name	Vector Backbone	Insert Description
pTM4	pFN11A	Sgfl-Pmel RNF8
pTM10	pFN10A	Sgfl-Pmel TUBEs
pTM20	pFN11A	Sgfl-Pmel RNF8 C403S
pTM40	pFN11A	Sgfl-Pmel CHFR
pTM48	pFN11A	Sgfl-Pmel TRAF6
pTM61	pFN11A	Sgfl-Pmel CHFR W240A
pTM63	pFN11A	Sgfl-Pmel TRAF6 R88A
pBIND	pFN11A	Empty control vector

pACT	pFN10A	Empty control vector
Reporter Vector	pGL4.31	Firefly Luciferase