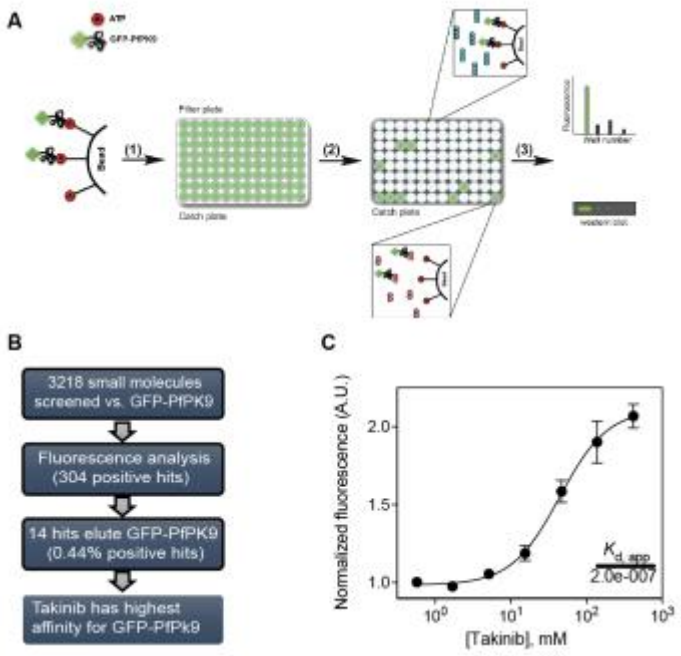


Figure S1. Identification of Takinib as an inhibitor of PfPK9 related to Figure 1. **A** Screening strategy for ATP pull-down assay. (1) ATP bound sepharose beads were incubated with cell lysate containing GFP-PfPK9. Following incubation, the charged ATP beads were dispensed into 96-well filter plates. (2) Small-molecules or ATP solutions were added to each well of 96-well filter plates. The plates were then incubated at room temperature and centrifuged to collect the eluates in the catch plate. (3) First, the fluorescence intensity of each eluate was measured using an Envision imager system. Next, each eluate with high fluorescence intensity (≥ 2-fold background fluorescence) was Western blotted for GFP-PfPK9 to confirm for hit selection. **B** Summary of screening results. **C** Affinity of Takinib for PfPK9 as determined in ATP pull-down assay. Kd,app calculated based on previous work(Haystead, 2006).

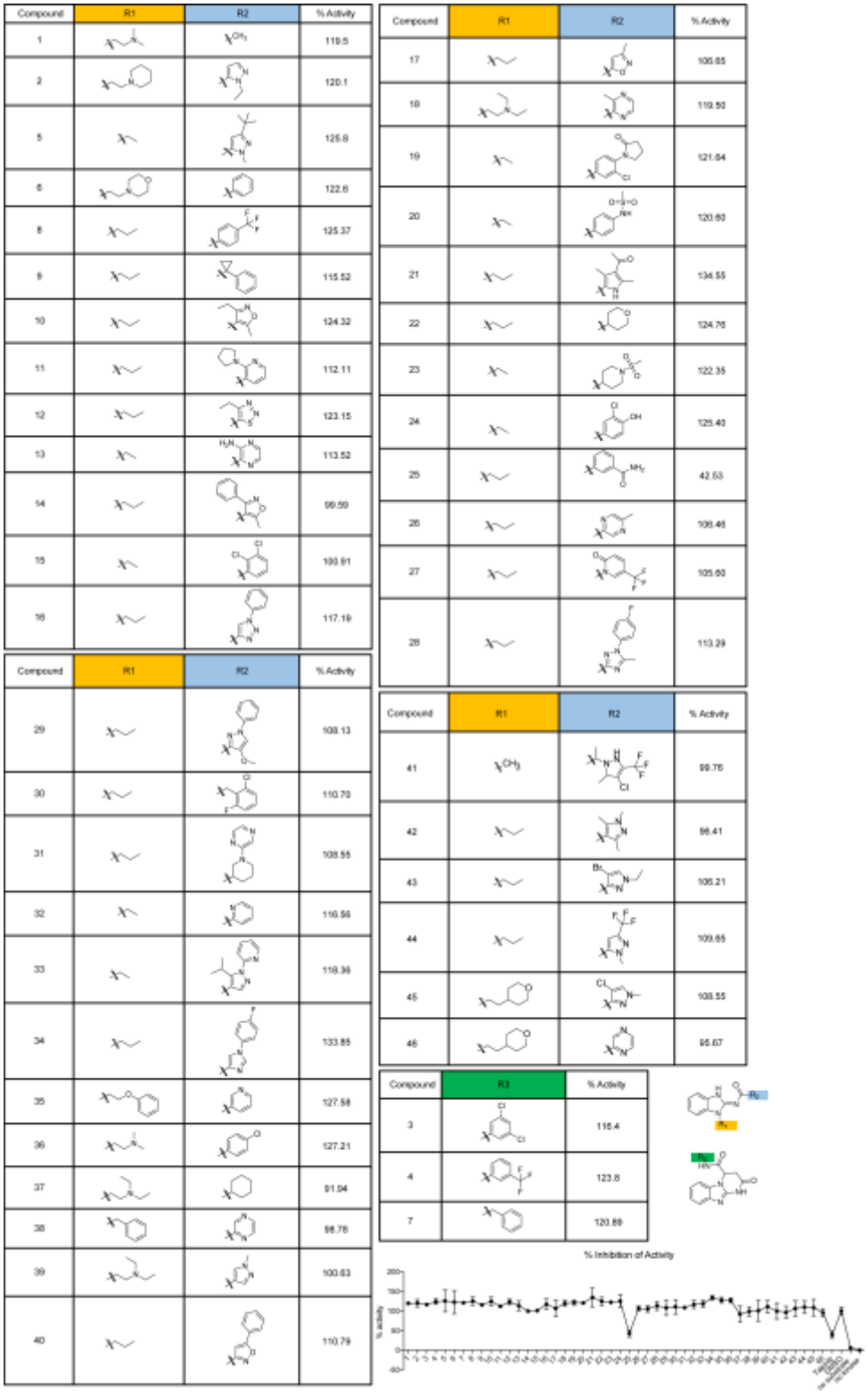


Figure S2. Structure-Activity Relationship Studies with Takinib and aminobenzimidazole containing analogs related to Figure 3. Table demonstrates analogs with graphical representation of results shown below. Analogs were taken from the Haystead library and numbers assigned by PFH. Experimenter JT was blinded for the kinase assay.

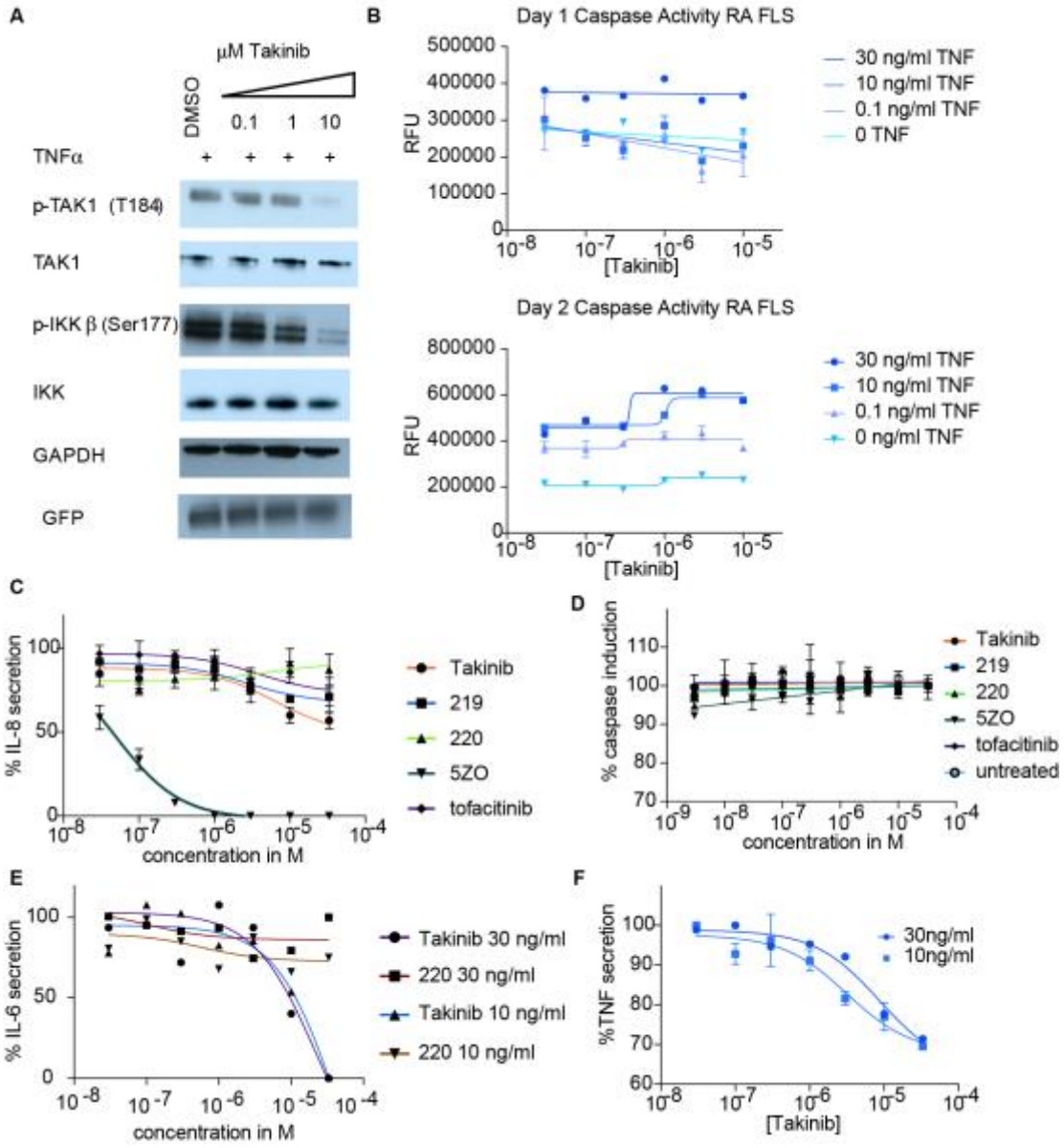


Figure S3. Additional Studies with RA FLS and cancer cells related to Figure 5, 6. **A** Caspase induction of RA FLS cells was measured after 24h treatment with Takinib compounds and TNF stimulation (n=4, mean±SEM). **B** As in **A,** but cells were treated for 48h (n=4, mean±SEM). **C** Caspase induction measured after 24h in the presence of TNF, cells treated with indicated compounds (n=4, mean±SEM). **D** IL-6 secretion was measured in the presence of different concentrations of TNF and Takinib /220 (n=2, mean±SEM). **E** IL8 secretion of cells treated with indicated compounds and TNF. Secretion was measured by performing ELISA (n=2, mean±SEM). **F** TNF secretion was measured after cells were stimulated with TNF in the presence of compounds. After 24h, media was changed to contain only compound. ELISA was used to measured TNF release of RA FLS (n=2, mean±SEM).

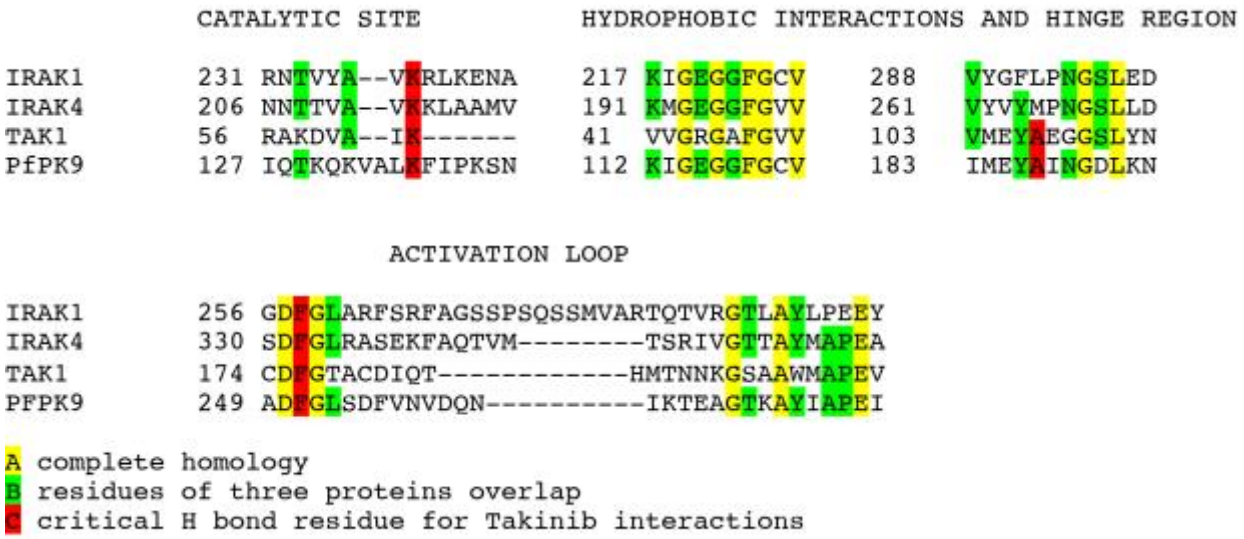
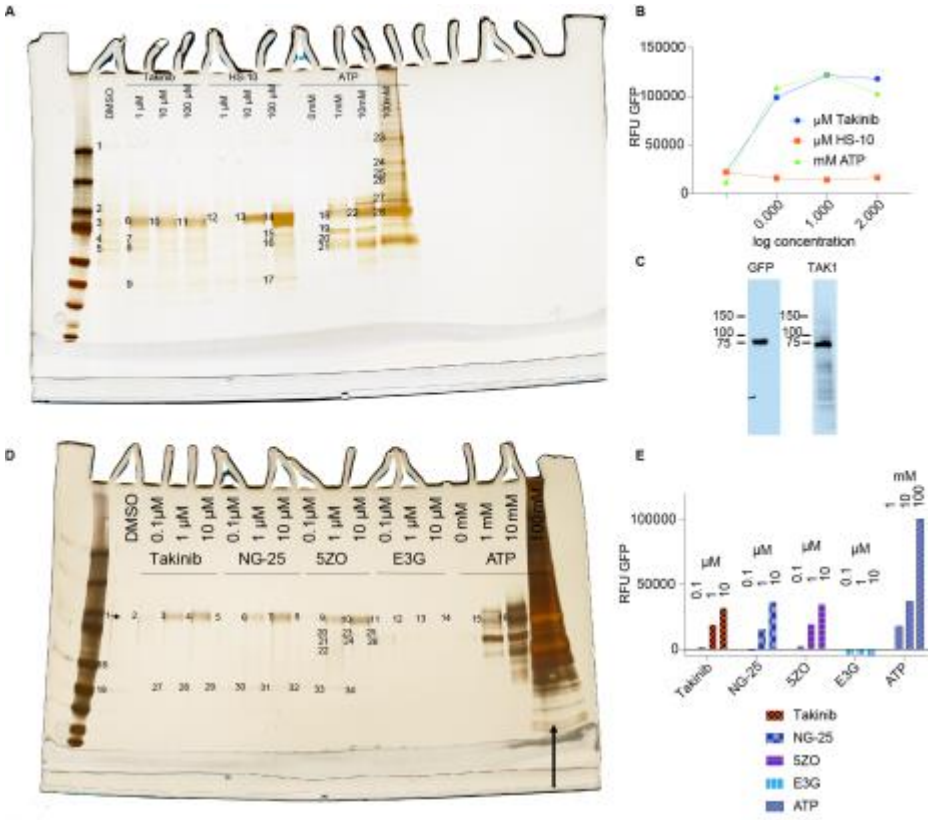


Figure S4. Sequence alignments of IRAK1, IRAK4, TAK1, and PfPK9 related to Figure 1. Sequences of TAK1 that are critical for Takinib binding were aligned with kinases for which Takinib significantly reduced activity in initial screens. Shown are catalytic site, hydrophobic interactions, the hinge region, and the activation loop for each kinase.



F

1	below C.I. threshold
2	below C.I. threshold
3	below C.I. threshold
4	below C.I. threshold
5	below C.I. threshold
6	TGF-beta-activated kinase 1 and MAP3K7-binding protein
7	HspTD
8	below C.I. threshold
9	40S ribosomal protein SA
10	Mitogen - activated protein kinase kinase kinase 7
11	Mitogen - activated protein kinase kinase kinase 7
12	Hsp90
13	Hsp90
14	Hsp90
15	Lysine-tRNA ligase
16	below C.I. threshold
17	40S ribosomal protein SA
18	Mitogen - activated protein kinase kinase kinase 7
19	HspTD
20	below C.I. threshold
21	T-complex protein 1 subunit zeta
22	Hsp90
23	Fatty acid synthase
24	Clathrin heavy chain
25	below C.I. threshold
26	below C.I. threshold
27	Splicing factor, proline- and glutamine-rich
28	Hsp90

G

1	below C.I. threshold
2	Mitogen-activated protein kinase kinase kinase 7
3	Mitogen-activated protein kinase kinase kinase 7
4	Mitogen-activated protein kinase kinase kinase 7
5	below C.I. threshold
6	Mitogen-activated protein kinase kinase kinase 7
7	Mitogen-activated protein kinase kinase kinase 7
8	Mitogen-activated protein kinase kinase kinase 7
9	Mitogen-activated protein kinase kinase kinase 7
10	Mitogen-activated protein kinase kinase kinase 7
11	below C.I. threshold
12	below C.I. threshold
13	Hsp90
14	below C.I. threshold
15	Mitogen-activated protein kinase kinase kinase 7
16	Hsp90
17	Hsp90
18	below C.I. threshold
19	40S ribosomal protein
20	Hsp70
21	below C.I. threshold
22	below C.I. threshold
23	Hsp70
24	below C.I. threshold
25	Hsp70
26	below C.I. threshold
27	below C.I. threshold
28	40S ribosomal protein
29	40S ribosomal protein
30	40S ribosomal protein
31	40S ribosomal protein
32	below C.I. threshold
33	Keratin
34	below C.I. threshold

H

100mM ATP lane
Keratin, type II cytoskeletal 2 epidermal
Cytoplasmic dynein 1 heavy chain
Nucleoprotein TPR
Spectrin beta chain, non erythrocytic
Fatty acid synthase
Myosin -10
Clathrin heavy chain 1
Clathrin heavy chain 1
Isoleucine-tRNA ligase
Splicing factor 3B subunit 3
ATP-citrate synthase
C-1-tetrahydrofolate synthase
Splicing factor, prolineand glutaminerich
Transitional endoplasmic reticulum ATPase
Heat shock protein HSP 90 beta
Heat shock protein 75 kDa, mitochondrial
Heat shock 70 kDa protein 1A/1B
T-complex protein 1 subunit gamma
T-complex protein 1 subunit alpha
Non-POU domaincontaining octamerbinding protein
Tubulin beta chain
Multifunctional protein ADE2
Multifunctional protein ADE2
Actin
40S ribosomal protein
Eukaryotic translation initiation factor 3 subunit I
Prohibitin-2
60S ribosomal protein L14
60S ribosomal protein L10a
40S ribosomal protein S5
40S ribosomal protein S18
60S ribosomal protein L27
40S ribosomal protein S19
Small nuclear ribonucleoprotein G

Figure S5. Purine-wide selectivity analysis of Takinib in comparison to TAK1 inhibitors related to Figure 1. **A** Silver-stained gel of small-molecule and ATP elutions of γ-linked ATP-sepharose resin from lysate expressing TAK1-TAB1-GFP. HS-10 (selective Hsp90 inhibitor) serves as a control **B** GFP fluorescence measured from elutions in A. **C** TAK1-TAB1-GFP expression determined by Western Blot. **D** As in A, elution profiles of TAK1 inhibitors and ATP, right table displays eluted proteins. Arrow indicates ATP lane sequenced by mass spec **E** GFP fluorescence measured from samples taken from C. **F**, **G**, **H** Tables of proteins identified by mass spec. Complete mass spec analysis shown in Table S3, S4.