Supplemental Table 1. Kinases where LY2228820 shows >1000-fold selectivity in vitro (p38α MAPK vs. other kinase)

ABL	CDK5/P35	FES	JAK3	MST1	PKCβ1	RSK3
ABL(T315I)	CDK6	FGFR1	KDR/VEGFR2	MST2	PKCβ2	SGK
AKT1	CDK7	FGFR2	KIT	MST3	PKCδ	SGK2
AKT2	CDK9	FGFR3	KIT(D816V)	MUSK	PKCε	SGK3
AKT3	CHK1	FGFR4	LCK	NEK11	PKCγ	SIK
ALK	CHK2	FGR	LKB1	NEK2	PKCη	SNK
ALK4	CK2	FLT1/VEGFR1	LIMK1	NEK3	PKCι	SRC
ARG	CK2α2	FLT3	LOK	NEK6	PKCμ	SRPK1
ARK5	CSK	FLT3(D835Y)	LYN	NEK7	PKCτ	SRPK2
ASK1	DAPK1	FLT4/VEGFR3	MK2	NLK	PKG1α	STK33
AUR2	DAPK2	FMS	MK3	P70S6	PKG1β	SYK
AXL	DMPK	FYN	MEK1	PAK2	PKX	TAK1
BMX	DRAK1	GSK3α	MELK	PAK3	PLK3	TBK1
BRK	DYRK2	GSK3β	cMET	PAK4	PRAK	TGFbR2
BRSK1	EEF2K	HCK	MINK	PAK5	PRK2	TIE2
BRSK2	EPHA1	HIPK1	MLCK	PAK6	PTK5	TRKA
BTK	EPHA2	HIPK2	MLK1	PAR1Bα	PYK2	TRKB
CAMKI	EPHA4	HIPK3	MLK2	PASK	RAF	TSSK1
CAMK2α	EPHA5	IKKα	MLK7	PDGFRα	RET	TSSK2
CAMK4	EPHA7	IKKβ	MNK2	PDGFRβ	ROCK1	WNK2
CDK1	EPHA8	IR	MRCKα	PDK1	ROCK2	WNK3
CDK2/CycA	EPHB2	IRAK1	MRCKβ	PIM1	RON	ZAP70
CDK2/CycE	EPHB3	IRAK4	MSK1	PIM2	RSE	ZIPK
CDK4	EPHB4	IRR	MSK2	PKAα	RSK1	YES
CDK5/P25	FER	ITK	MSSK1	PKCα	RSK2	

Assays were configured in either 96-well radiometric filter binding (^{33}P-ATP phospho-cellulose or glass fiber) or fluorescence polarization formats under linear velocity conditions, at or below the Km[ATP] (30-120 minutes, $\leq$ 10% ATP conversion) using 1-4% DMSO final. IC50 values were calculated from 10-point concentration-response curves (1:3 serial dilutions from 20 μM to 1 nM) using a 4-parameter non-linear regression fit (ActivityBase, IDBS).

Supplemental Figure legends

Supplemental Figure 1. A) Effect of LY2228820 on MK2 phosphorylation in mouse peripheral blood mononuclear cells (PBMC). PBMC's were collected from mice treated with LY2228820 dosed orally (0.1 → 30 mg/kg p.o.) and blood collected 2 hours later. FITC-conjugated rat anti-mouse Ly-6G, APC-conjugated rat anti-mouse CD11b, and anisomycin (10 μg/mL) were added to 100 μL blood. Following addition of Lyse/Fix buffer, white blood cells were collected by centrifugation and washed. Cells were resuspended and stained with rabbit anti-p-MK2 (p-Thr334). Following centrifugation and washing, cells were resuspended in staining/washing buffer containing anti-rabbit Ig-PE conjugate. Cells were again centrifuged and washed 2X. Stained cells were collected and analyzed by flow cytometry (Beckman F500). LY2228820 effectively inhibited mouse MK2 phosphorylation: threshold ED_{50} = 1.01 mg/kg [compound exposure approx. 100 nM]. **B) PBMC p-MK2 from patients with multiple myeloma treated with LY2228820 ex vivo.** Samples of peripheral blood from 4 multiple myeloma patients were collected. Samples were incubated with LY2228820 (ranging from 100 → 0.03 μM, eight 1:3 serial dilutions) or buffer control. Cells were labeled with CD14-FITC $\pm$ anisomycin (2ug/ml), lysed and simultaneously fixed. Following a wash step, samples were labeled with p-Thr^{334}-MK2 Ab for 2hrs, washed, and secondary goat anti-rabbit Ig-R-PE added. Samples were washed and fixed with 1% paraformaldehyde for flow cytometric analysis. From the FL1 (FITC) vs. side scatter (SS) plot, the monocytic population was selected using the CD14-FITC Ab and gated. Using this gating, the PE fluorescence was measured. LY2228820 potently inhibited p-MK2 by human PBMC (IC_{50} = 0.12 μM). Detailed methods are described in Zhao J et al., Rapid and quantitative detection of p38 kinase pathway in mouse blood monocyte. In Vitro Cell Dev Biol Anim. 2008; 44:145-53.

Supplemental Figure 2. shRNA silencing of p38α MAPK (p38α) in U-87MG glioma

shRNA silencing of p38 alpha was analyzed for protein expression (p38 MAPK alpha, Cell Signaling #9226) in wild type U-87MG cells ("parental", black line), U-87MG cells stably expressing an shRNA against green fluorescent protein ("shGFP"; essentially a non-targeting control shRNA) or U-87MG cells stably expressing an shRNA against p38 MAPK alpha ("p38α KD", as described under "Methods"). Tumor cells were implanted subcutaneously into the right rear flank of athymic female nude mice and growth was monitored over a period of ~ 6 weeks. *Inset*; Western blot of total p38 MAPK alpha and the downstream signaling protein phospho MAPKAPK2 (p-MK2) from lentivirally transduced U-87MG cells described above, as described under "Methods." Note that there is a nearly complete reduction in total p38 MAPK alpha in the cells stably expressing the specific shRNA against this target, and a corresponding reduction in p-MK2.

Supplemental Methods (Suppl. Figure 1).

Phosphorylation of MK2 in mouse peripheral blood mononuclear cells (PBMC): Female BALB/c mice were purchased from Taconics (Indianapolis) and maintained as described below (In vivo animal care). Animals were dosed with LY2228820 (0.1→30 mg/kg p.o.) in 1% carboxymethylcellulose (CMC)/0.25% Tween® 80. Mouse whole blood was collected 2 hours after LY228820 administration via cardiac puncture in heparinized tubes (BD Vacutainer). FITC-conjugated rat anti-mouse Ly-6G (1:250, BD Biosciences), APC-conjugated rat anti-mouse CD11b (1:100, BD Biosciences), and anisomycin (10 µg/mL, Sigma) were added to 100 µL blood and incubated at 37 °C for 15 minutes. Lyse/Fix buffer (20X vol:vol, BD Phosflow) was added and vortexed. White blood cells were collected by centrifugation and then washed with staining/washing buffer (PBS w/0.09% NaN3 and 5% FBS, GIBCO). Cells were resuspended in 200 µL permeabilization medium B (Caltag Inc.) with rabbit anti-p-MK2 (p-Thr334, Cell Signaling Technologies) and incubated at room temp for 30 minutes. Following centrifugation and washing (2X with staining/washing buffer), cells were resuspended in staining/washing buffer containing anti-rabbit Ig-PE conjugate (1:250, BD Biosciences) and incubated at room temp for 30 minutes. Cells were again centrifuged and washed 2X. Stained cells were collected and analyzed by flow cytometry (Beckman F500, Beckman Inc.).

Phosphorylation of MK2 in human peripheral blood mononuclear cells (PBMC): Samples of peripheral blood from 4 multiple myeloma patients were collected in ACD solution B Vacutainers (Becton Dickinson), inverted several times to mix, and held at 4 °C overnight to simulate transport time from clinical site to lab. Cells were warmed to room temperature and then dispersed into tubes. Samples were then incubated with LY2228820 (ranging from 100 → 0.03 µM, eight 1:3 serial dilutions) or buffer control. Samples were labeled with CD14-FITC $\pm$ anisomycin (2 µg/mL), lysed and simultaneously fixed. After a single wash, samples were exposed to cold 100% MeOH and held at 4 °C for 30 minutes. Following a wash step, samples were labeled with p-Thr^{334}-MK2 Ab (Cell Signaling Technologies) for approx. 2 hours, washed, and secondary goat anti-rabbit Ig-R-PE (BD Biosciences) added for

30 minutes at room temp. Samples were washed and fixed with 1% paraformaldehyde for flow cytometric analysis. From the FL1 (FITC) vs. side scatter (SS) plot, the monocytic population was selected using the CD14-FITC Ab (BD Biosciences) and gated. Using this gating, the PE fluorescence was measured.

Supplemental Methods (Suppl. Figure 2).

shRNA knockdown: Lentivirus was purchased from Sigma-Aldrich. For p38 MAPK alpha the following clones against gene accession number NM_001315 were pooled: TRCN0000000509, TRCN0000000510, TRCN000000051, TRCN0000000512 and TRCN0000000513. Final titer was 1.01e7 TU/ml. For shGFP cat# SHC004V was used per manufacturer's specifications. Final multiplicity of infection for both treatments was ~17. Cells were plated in 24-well dishes at 25K/well and allowed to attach. Twenty-four hours post-plating, growth media was removed and replaced with media containing 8 µg/mL protamine sulfate (Sigma-Aldrich # P4020) and shRNA at a total volume of 500 µL/well. An additional 500 µL/well fresh media was added 24 hour post-infection. 48 hour post-infection media was aspirated and replaced with fresh media containing 2 µg/mL puromycin (Sigma-Aldrich # P9620). Selection continued for one week until a puromycin resistant population was confirmed. For Western blotting of p38 MAPK alpha and phospho-MAPKAPK2, cells were lysed in 1% SDS and analyzed for protein expression (p38 MAPK alpha, Cell Signaling #9228; phospho-MAPKAPK2, Epitomics # 1983-1).