

Supplementary Materials for

A highly selective JAK3 inhibitor is developed for treating rheumatoid arthritis by suppressing γ c cytokine-related JAK-STAT signal

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Supplementary Text

Stability of Z583 in hepatocytes

The hepatocytes of human (from 10 Donor Pooled), rat (male CD-1), and dog (male Beagle) were used to perform the stability of Z583 by Pharmaron Inc., (Beijing, China). Briefly, prepare 10 mM stock solutions of Z583 and positive control in appropriate solvent (DMSO). In separate conical tubes, dilute the 10 mM test compound and the positive control to 100 μ M by combining 495 μ l of 50% acetonitrile/50% water and 5 μ l of 10 mM stock. Place incubation medium (William's E Medium supplemented with GlutaMAX) and hepatocyte thawing medium in a 37°C water bath, and allow warming for at least 15 min prior to use. Remove a vial of cryopreserved hepatocytes from storage, ensuring that vials remain at cryogenic temperatures until thawing process ensues. Thaw the cells by placing the vial in a 37°C water bath and gently shaking the vials for 2 minutes. After thawing is completed, spray vial with 70% ethanol, transfer the vial to a biosafety cabinet. Dilute cells with incubation medium to a working cell density of 0.5×10^6 viable cells/ml. A portion of the hepatocytes at 0.5×10^6 viable cells/ml should be boiled for 5 min prior to adding to the plate as representative of a negative control. Place the plate in the incubator on an orbital shaker to allow the hepatocytes to warm for 10 min. Remove well contents in 25 μ l aliquots at time points of 0, 15, 30, 60, 90 and 120 min. The aliquots are then mixed with 6 volumes (150 μ l) of acetonitrile containing internal standards (IS: 100 nM alprazolam, 200 nM labetalol, 200 nM caffeine and 2 μ M ketoprofen) to terminate the reaction. Centrifuge the plate for 20 minutes at 3,220 g. Aliquot of 100 μ l of the supernatant was mixed with 100 μ l of ultra-pure H₂O and then used for LC-MS/MS analysis. All incubations will be performed in duplicate. All calculations were carried out using Microsoft Excel. Peak areas were determined from extracted ion chromatograms. Determine the *in vitro* half-life ($t_{1/2}$) of parent compound by regression analysis of the percent parent disappearance vs. time curve. The *in vitro* half-life (*in vitro* $t_{1/2}$) was determined from the slope value: *in vitro* $t_{1/2} = 0.693 / k$. Conversion of the *in vitro* $t_{1/2}$ (in min) into the scale-up intrinsic clearance (Scaled-up CL_{int}, in ml/min/kg) was done using the following equation (mean of duplicate determinations):

Scaled-up CL_{int} = $kV/N \times$ scaling factor (V: incubation volume, 0.2 ml; N: number of hepatocytes per well, 0.1×10^6 cells).

Stability of Z583 in Whole Blood

The whole blood of human (from health Mongloid volunteers, collected local hospital with Ethical approval, gender mixed), Rat (CD-1, gender mixed), and dog (Beagle, gender mixed) was used to perform the stability of Z583 by Pharmaron Inc., (Beijing, China). Briefly, 1 mM working solution of Z583 was prepared in DMSO, 1 mM working solution of control compound propantheline was prepared in acetonitrile. 4 μ l of working solutions was spiked to 796 μ l of pre-incubated whole blood to reach a final concentration of 5 μ M. The final concentration of solvent was 0.5%. 50 μ l aliquots of the spiked whole blood were added into new tubes for different time points including 15, 30, 60 and 120 min and incubated at 37°C water bath with shaking at 60 rpm. The assay was performed in duplicate. The reaction was stopped by adding 300 μ l of room temperature quench solution (acetonitrile containing internal standards (IS, 100 nM Alprazolam, 500 nM Labetalol and 2 μ M Ketoprofen) to the spiked whole blood samples at the appointed time points. Time 0 samples were prepared by adding 50 μ l of the spiked whole blood to new tubes containing 300 μ l of room temperature quench solution. Vortex for 5 min and samples in plate were centrifuged at 3,220 g for 30 min at 4°C to precipitate protein. And then 100 μ l of the supernatant was transferred to a new 96-well plate with 100 μ l water for LC-MS/MS analysis. All calculations were carried out using Microsoft Excel. Peak area ratios were determined from extracted ion chromatograms. Percent compounds remaining at each time point were calculated by the following equation:

$$\text{Remaining Percentage t min (\%)} = \text{Peak Area Ratio t min} / \text{Peak Area Ratio 0 min} \times 100$$

Where Peak Area Ratio t min is peak area ratio of control and test compound at t min;

Peak Area Ratio 0 min is peak area ratio of control and test compound at zero time point.

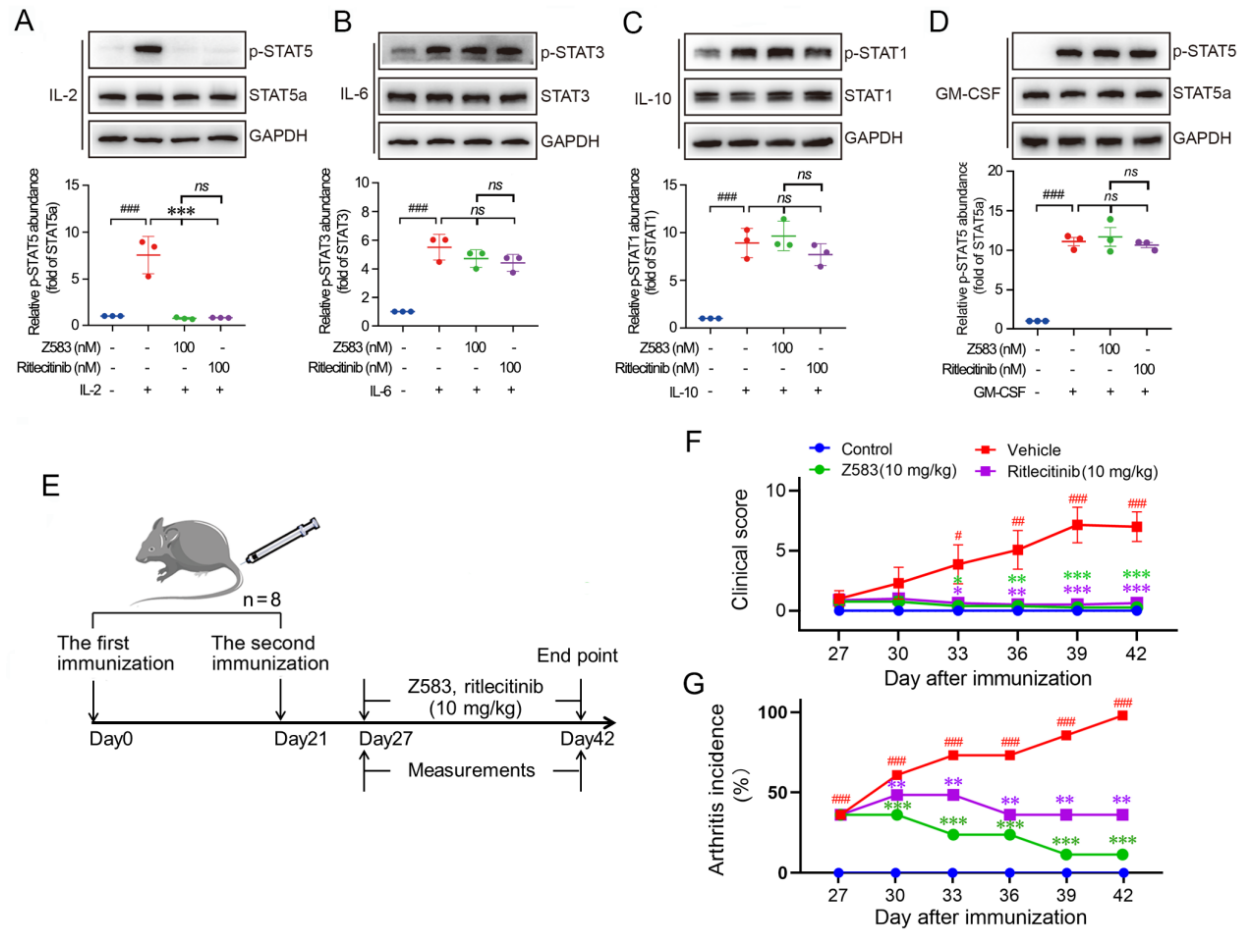


Fig S1. The effects of Z583 and ritlecitinib *in vitro* signaling level and RA treatment *in vivo*. Human PBMCs with or without Z583 and ritlecitinib were stimulated with γ c-associated IL-2 (A) and other cytokines (B-D). All data are from three independent experiments. Data are presented as means \pm SEM. $####P < 0.001$ versus control group; $***P < 0.001$ versus alone cytokine-stimulating group; and *ns*, no statistical significance. (E) Experimental scheme of Z583 and ritlecitinib for the treatment of CIA. Clinical score (F) and CIA incidence (G) were assessed every three days in control, CIA mice, Z583-treated CIA mice, and ritlecitinib-treated CIA mice. Data are presented as means \pm SEM, $n = 8$. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ versus control group; $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus CIA group.

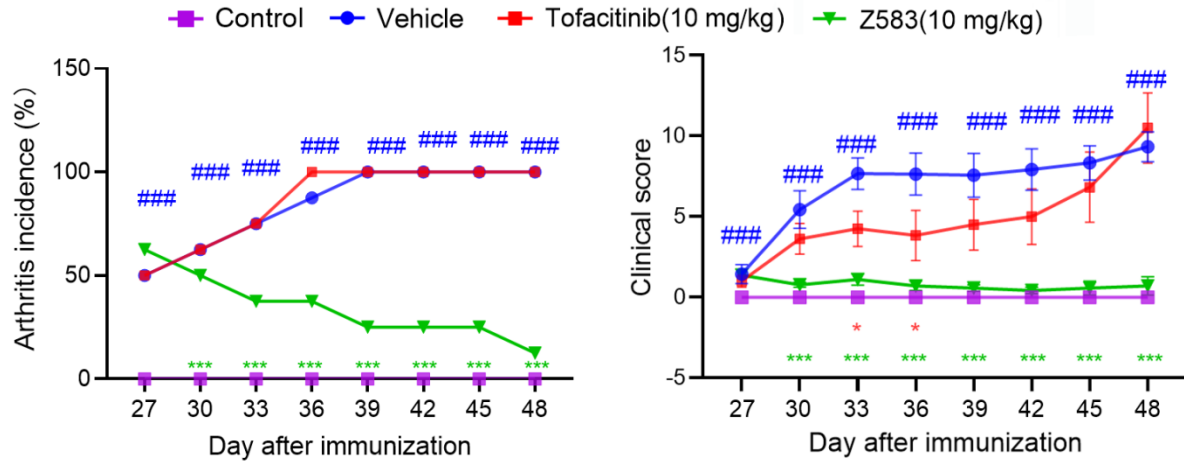


Fig. S2. Prophylactic effect of Z583 and tofacitinib in CIA mice. Clinical score and CIA incidence were assessed every three days in control, vehicle CIA mice, tofacitinib-treated CIA mice, and Z583-treated CIA mice. Data are presented as means \pm SEM, $n = 8$. ### $P < 0.001$ versus control group; *** $P < 0.001$ versus CIA group.

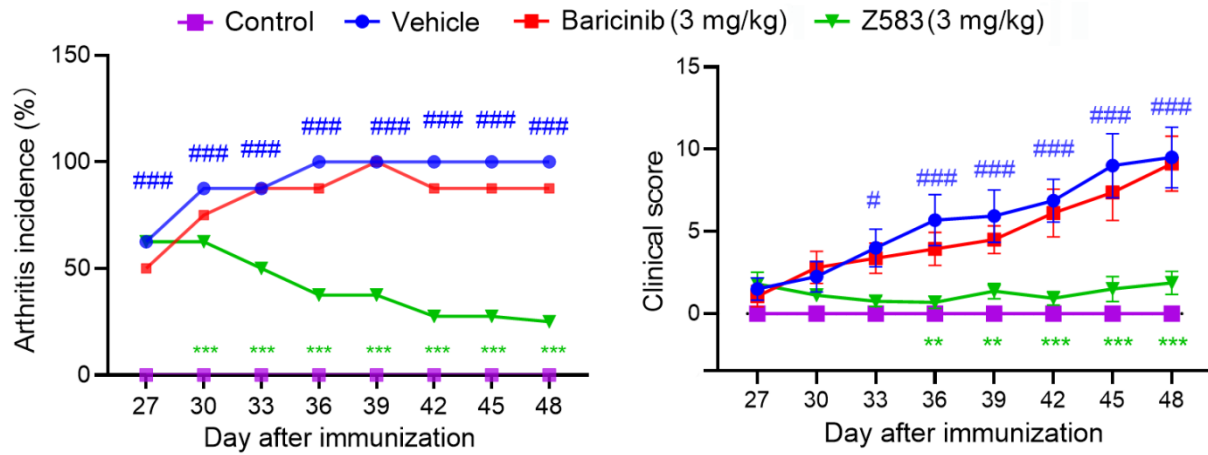


Fig. S3. Prophylactic effect of Z583 and baricininib in CIA mice. Clinical score and CIA incidence were assessed every three days in control, vehicle CIA mice, baricininib-treated CIA mice, and Z583-treated CIA mice. Data are presented as means \pm SEM, $n = 8$. # $P < 0.05$, and ### $P < 0.001$ versus control group; * $P < 0.01$, and *** $P < 0.001$ versus CIA group.

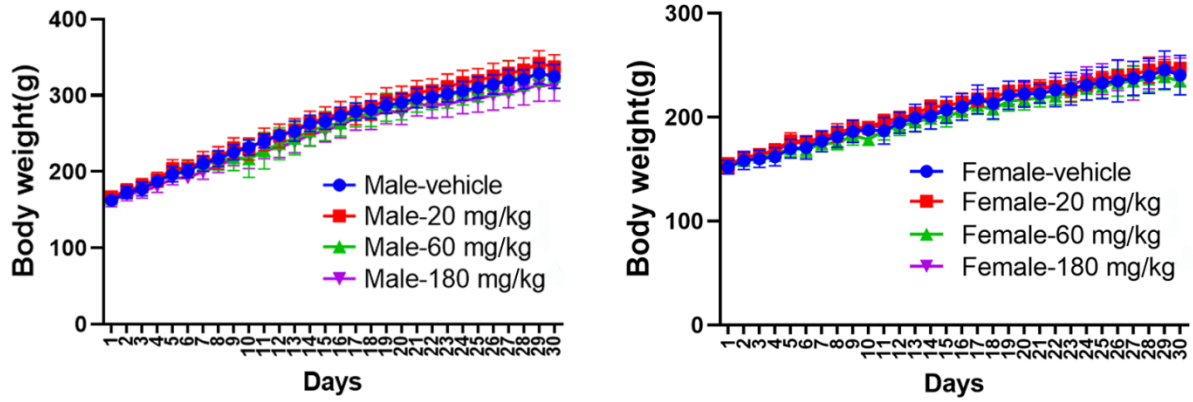


Fig. S4. The changes of body weight of rats in subacute toxicity analysis. The body weight of male- and female-rats were measured after treating with Z583 (20, 60, and 180 mg/kg, p.o.) for four weeks.

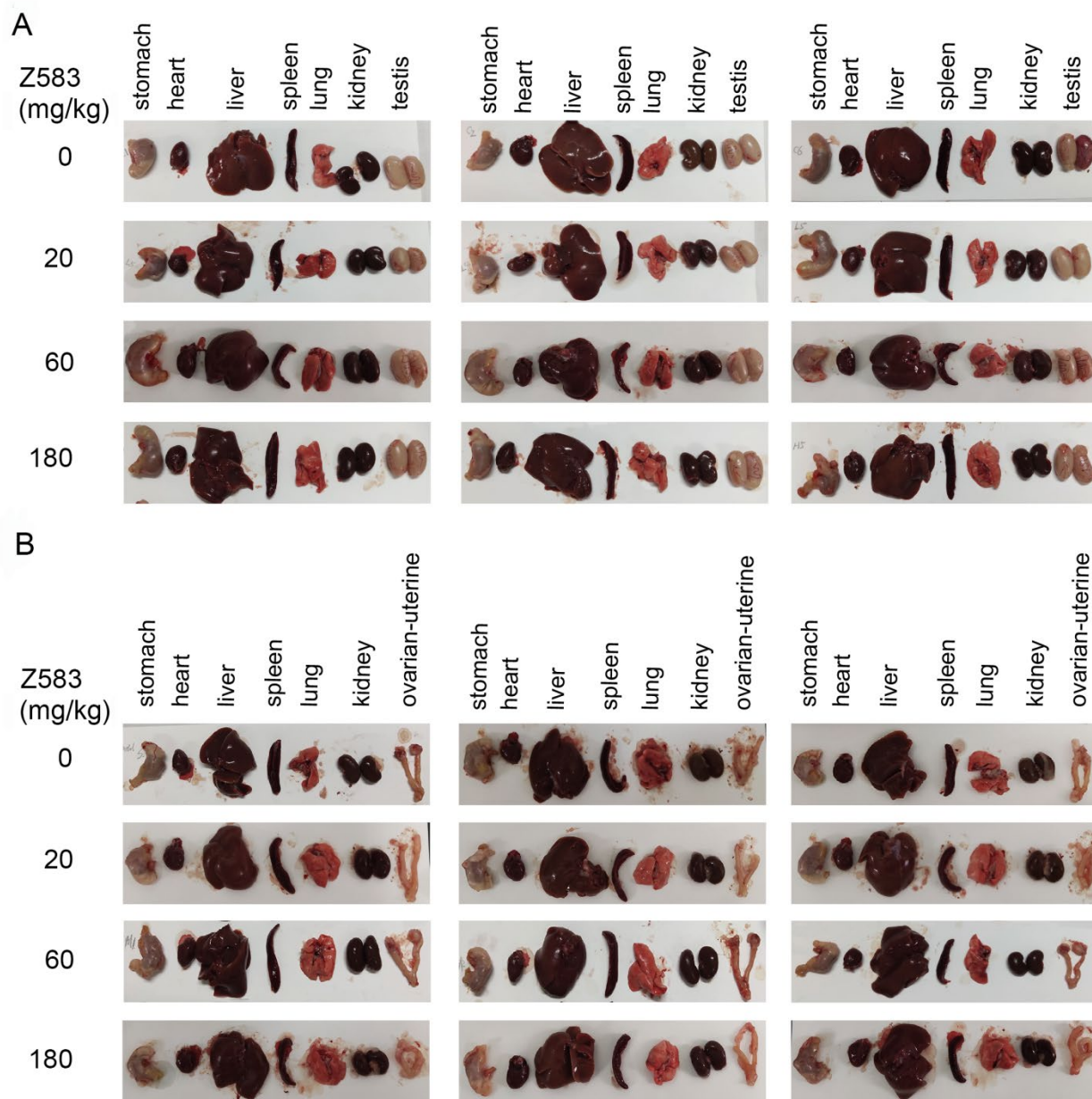


Fig. S5. Represented images of organs from subacute toxicity experiment. Macroscopic images obtained from heart, liver, spleen, lung, kidney stomach, testis, ovarian and uterine of (A) male and (B) female rat after treating with Z583 (20, 60, and 180 mg/kg , p.o.) for four weeks.

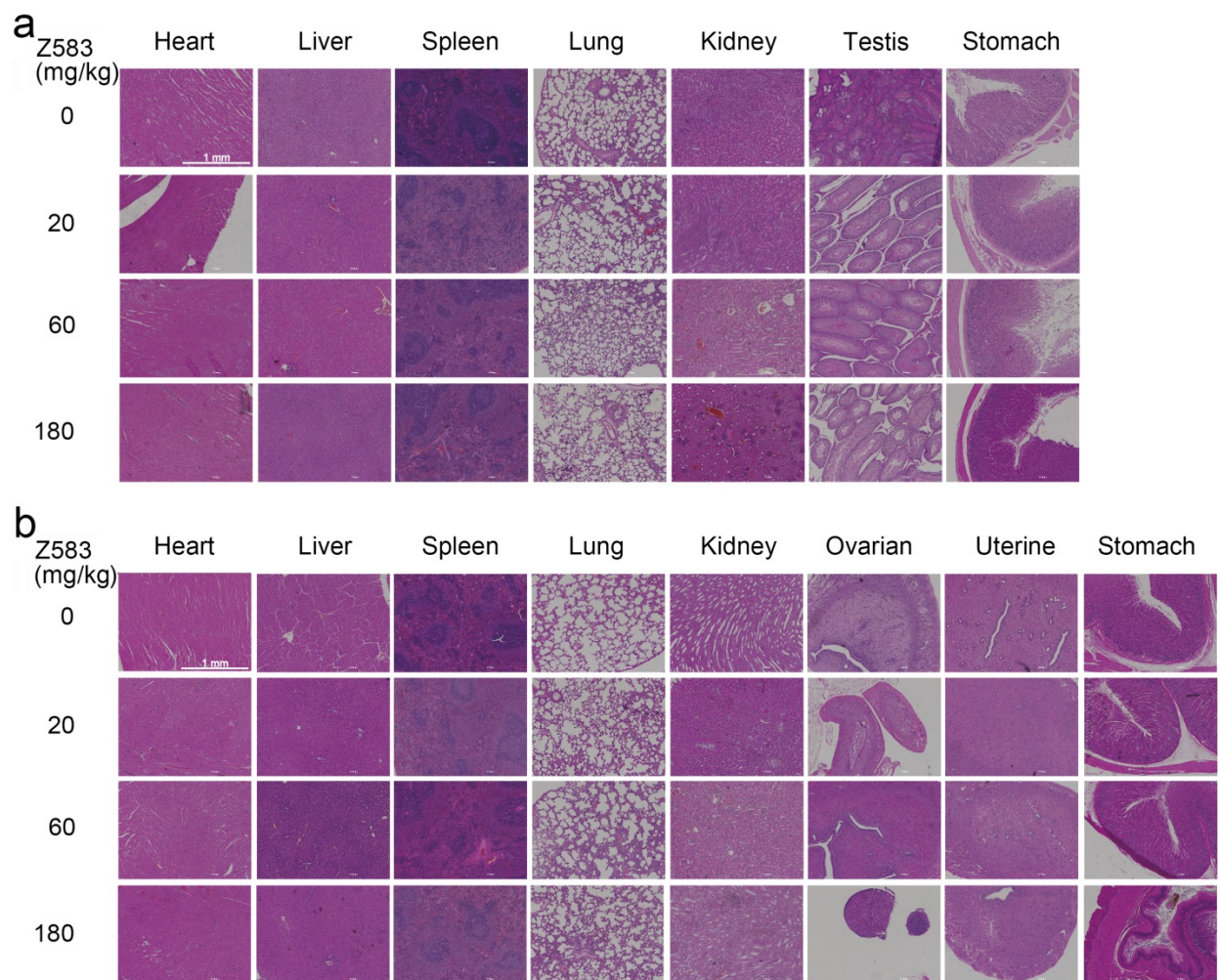


Fig. S6. Histological images of organs from subacute toxicity experiment. Organs stained by H&E obtained from heart, liver, spleen, lung, kidney stomach, testis, ovarian and uterine of (A) male and (B) female rat after treating with Z583 (20, 60, and 180 mg/kg, p.o.) for four weeks.

Table S1. 463 kinase selectivity screening of Z583 at a concentration of 100 nM (Discover X).

Kinase Tested	Percent Control	Kinase Tested	Percent Control
AAK1	7.6	MAP3K15	94
ABL1(E255K)-phosphorylated	6.1	MAP3K2	80
ABL1(F317I)-nonphosphorylated	77	MAP3K3	100
ABL1(F317I)-phosphorylated	69	MAP3K4	100
ABL1(F317L)-nonphosphorylated	17	MAP4K2	48
ABL1(F317L)-phosphorylated	24	MAP4K3	100
ABL1(H396P)-phosphorylated	8.2	MAP4K4	78
ABL1(M351T)-phosphorylated	8.5	MAP4K5	93
ABL1(Q252H)-nonphosphorylated	14	MAPKAPK2	100
ABL1(Q252H)-phosphorylated	8.9	MAPKAPK5	96
ABL1(T315I)-nonphosphorylated	16	MARK1	85
ABL1(T315I)-phosphorylated	14	MARK2	100
ABL1(Y253F)-phosphorylated	7.7	MARK3	100
ABL1-nonphosphorylated	2.1	MARK4	85
ABL1-phosphorylated	9.3	MAST1	100
ABL2	15	MEK1	100
ACVR1	97	MEK2	99
ACVR1B	93	MEK3	75
ACVR2A	98	MEK4	100
ACVR2B	87	MEK5	80
ACVRL1	100	MEK6	86
ADCK3	100	MELK	71
ADCK4	100	MERTK	100
AKT1	100	MET	57
AKT2	100	MET(M1250T)	73
AKT3	100	MET(Y1235D)	100
ALK	80	MINK	84
ALK(C1156Y)	77	MKK7	80
ALK(L1196M)	89	MKNK1	84
AMPK-alpha1	100	MKNK2	22
AMPK-alpha2	100	MLCK	81
ANKK1	100	MLK1	51
ARK5	37	MLK2	56
ASK1	100	MLK3	87
ASK2	92	MRCKA	100
AURKC	0.55	MRCKB	100
AXL	100	MST1	100
BIKE	3.3	MST1R	100
BLK	1.6	MST2	82

BMPR1A	88	MST3	100
BMPR1B	67	MST4	100
BMPR2	90	MTOR	100
BMX	0	MUSK	94
BRAF	98	MYLK	100
BRAF(V600E)	89	MYLK2	67
BRK	97	MYLK4	85
BRSK1	100	MYO3A	100
BRSK2	100	MYO3B	57
BTK	0	NDR1	100
BUB1	86	NDR2	100
CAMK1	100	NEK1	100
CAMK1B	92	NEK10	91
CAMK1D	100	NEK11	96
CAMK1G	97	NEK2	100
CAMK2A	100	NEK3	67
CAMK2B	99	NEK4	100
CAMK2D	100	NEK5	57
CAMK2G	100	NEK6	100
CAMK4	100	NEK7	97
CAMKK1	95	NEK9	99
CAMKK2	76	NIK	69
CASK	99	NIM1	98
CDC2L1	100	NLK	100
CDC2L2	100	OSR1	93
CDC2L5	79	p38-alpha	100
CDK11	100	p38-beta	98
CDK2	85	p38-delta	100
CDK3	88	p38-gamma	86
CDK4	97	PAK1	100
CDK4-cyclinD1	78	PAK2	83
CDK4-cyclinD3	75	PAK3	95
CDK5	97	PAK4	100
CDK7	55	PAK6	98
CDK8	93	PAK7	100
CDK9	100	PCK1	86
CDKL1	85	PCK2	100
CDKL2	69	PCK3	100
CDKL3	77	PDGFRA	69
CDKL5	100	PDGFRB	17
CHEK1	100	PDPK1	75
CHEK2	88	PFCDPK1(P.falciparum)	100
CIT	80	PFPK5(P.falciparum)	87
CLK1	91	PFTAIRE2	100
CLK2	32	PFTK1	100
CLK3	100	PHKG1	100

CLK4	100	PHKG2	100
CSF1R	44	PIK3C2B	79
CSF1R-autoinhibited	3.4	PIK3C2G	72
CSK	100	PIK3CA	80
CSNK1A1	90	PIK3CA(C420R)	82
CSNK1A1L	100	PIK3CA(E542K)	100
CSNK1D	100	PIK3CA(E545A)	87
CSNK1E	100	PIK3CA(E545K)	100
CSNK1G1	100	PIK3CA(H1047L)	97
CSNK1G2	94	PIK3CA(H1047Y)	87
CSNK1G3	100	PIK3CA(I800L)	100
CSNK2A1	70	PIK3CA(M1043I)	67
CSNK2A2	23	PIK3CA(Q546K)	97
CTK	100	PIK3CB	100
DAPK1	94	PIK3CD	62
DAPK2	100	PIK3CG	26
DAPK3	100	PIK4CB	72
DCAMKL1	99	PIKFYVE	72
DCAMKL2	100	PIM1	100
DCAMKL3	100	PIM2	100
DDR1	100	PIM3	99
DDR2	80	PIP5K1A	3.3
DLK	93	PIP5K1C	54
DMPK	89	PIP5K2B	8.2
DMPK2	96	PIP5K2C	29
DRAK1	2	PKAC-alpha	100
DRAK2	1.8	PKAC-beta	100
DYRK1A	100	PKMYT1	100
DYRK1B	85	PKN1	100
DYRK2	100	PKN2	42
EGFR	100	PKNB(M.tuberculosis)	56
EGFR(E746-A750del)	90	PLK1	96
EGFR(G719C)	35	PLK2	100
EGFR(G719S)	43	PLK3	100
EGFR(L747-E749del, A750P)	91	PLK4	11
EGFR(L747-S752del, P753S)	87	PRKCD	100
EGFR(L747-T751del,Sins)	100	PRKCE	100
EGFR(L858R)	88	PRKCH	100
EGFR(L858R,T790M)	21	PRKCI	72
EGFR(L861Q)	68	PRKCQ	100
EGFR(S752-I759del)	94	PRKD1	100
EGFR(T790M)	28	PRKD2	100
EIF2AK1	85	PRKD3	100
EPHA1	31	PRKG1	100
EPHA2	90	PRKG2	100
EPHA3	80	PRKR	99

EPHA4	100	PRKX	100
EPHA5	80	PRP4	23
EPHA6	100	PYK2	100
EPHA7	100	QSK	100
EPHA8	100	RAF1	100
EPHB1	86	RET	67
EPHB2	87	RET(M918T)	67
EPHB3	100	RET(V804L)	91
EPHB4	94	RET(V804M)	73
EPHB6	29	RIOK1	13
ERBB2	88	RIOK2	67
ERBB3	100	RIOK3	4.5
ERBB4	43	RIPK1	92
ERK1	78	RIPK2	94
ERK2	86	RIPK4	87
ERK3	94	RIPK5	88
ERK4	100	ROCK1	74
ERK5	100	ROCK2	47
ERK8	27	ROS1	61
ERN1	100	RPS6KA4(Kin.Dom.1-N-terminal)	100
FAK	100	RPS6KA4(Kin.Dom.2-C-terminal)	86
FER	100	RPS6KA5(Kin.Dom.1-N-terminal)	100
FES	100	RPS6KA5(Kin.Dom.2-C-terminal)	99
FGFR1	27	RSK1(Kin.Dom.1-N-terminal)	100
FGFR2	27	RSK1(Kin.Dom.2-C-terminal)	93
FGFR3	43	RSK2(Kin.Dom.1-N-terminal)	95
FGFR3(G697C)	39	RSK2(Kin.Dom.2-C-terminal)	96
FGFR4	83	RSK3(Kin.Dom.1-N-terminal)	100
FGR	35	RSK3(Kin.Dom.2-C-terminal)	99
FLT1	29	RSK4(Kin.Dom.1-N-terminal)	82
FLT3	12	RSK4(Kin.Dom.2-C-terminal)	100
FLT3(D835H)	15	S6K1	90
FLT3(D835Y)	33	SBK1	90
FLT3(ITD)	38	SGK	75

FLT3(ITD,D835V)	26	SgK110	21
KIT(V559D,T670I)	55	SGK2	76
FLT3(ITD,F691L)	9.6	SGK3	96
FLT3(K663Q)	46	SIK	85
FLT3(N841I)	3.2	SIK2	81
FLT3(R834Q)	20	SLK	84
FLT3-autoinhibited	75	SNARK	20
FLT4	26	SNRK	89
FRK	52	SRC	26
FYN	100	SRMS	100
GAK	95	SRPK1	11
GCN2(Kin.Dom.2,S808G)	100	SRPK2	65
GRK1	100	SRPK3	16
GRK2	100	STK16	19
GRK3	91	STK33	100
GRK4	11	STK35	100
GRK7	97	STK36	94
GSK3A	100	STK39	100
GSK3B	100	SYK	100
HASPIN	74	TAK1	79
HCK	100	TAOK1	27
HIPK1	88	TAOK2	82
HIPK2	84	TAOK3	44
HIPK3	100	TBK1	27
HIPK4	95	TEC	4.1
HPK1	54	TESK1	100
HUNK	100	TGFBR1	100
ICK	46	TGFBR2	100
IGF1R	98	TIE1	21
IKK-alpha	100	TIE2	92
IKK-beta	98	TLK1	100
IKK-epsilon	50	TLK2	100
INSR	87	TNIK	100
INSRR	98	TNK1	5.5
IRAK1	74	TNK2	73
IRAK3	75	TNNI3K	97
IRAK4	95	TRKA	40
ITK	31	TRKB	56
JAK1(JH1domain-catalytic)	71	TRKC	70
JAK1(JH2domain-pseudokinase)	80	TRPM6	54
JAK2(JH1domain-catalytic)	4.1	TSSK1B	100
JAK3(JH1domain-catalytic)	0.35	TSSK3	94
JNK1	72	TTK	59
JNK2	88	TXK	0.3
JNK3	73	TYK2(JH1domain-catalytic)	16

KIT	29	TYRO3	83
KIT(A829P)	5.4	ULK1	63
KIT(D816H)	22	ULK2	97
KIT(D816V)	21	ULK3	77
KIT(L576P)	12	VEGFR2	16
KIT(V559D)	20	VPS34	21
KIT(V559D,V654A)	76	VRK2	76
KIT-autoinhibited	66	WEE1	100
LATS1	68	WEE2	100
LATS2	83	WNK1	100
LCK	95	WNK2	90
LIMK1	45	WNK3	96
LIMK2	91	WNK4	91
LKB1	61	YANK1	84
LOK	100	YANK2	100
LRRK2	64	YANK3	100
LRRK2(G2019S)	36	YES	43
LTK	100	YSK1	100
LYN	81	YSK4	1.4
LZK	100	ZAK	99
MAK	97	ZAP70	54
MAP3K1	100		

Note: Data for binding interactions are reported as %control, where lower numbers indicate stronger hits in the matrix.

Table S2. The hematological index of rats in subacute toxicity assay.

Day 0	Male (vehicle)	Male (20 mg/kg)	Male (60 mg/kg)	Male (180 mg/kg)	Female (vehicle)	Female (20 mg/kg)	Female (60 mg/kg)	Female (180 mg/kg)
WBC (10 ⁹ /l)	11.22±3.35	10.48±1.62	9.81±5.54	9.42±1.20	10.31±1.29	10.42±2.09	12.61±1.28	8.72±2.49
LYM (10 ⁹ /l)	8.66±2.74	8.41±1.40	7.56±4.35	7.39±1.30	8.40±1.47	8.43±1.81	9.88±0.78	7.42±2.14
MONO(10 ⁹ /l)	0.66±0.15	0.50±0.08	0.56±0.34	0.61±0.19	0.53±0.08	0.50±0.14	0.49±0.13	0.30±0.09
NEUT(10 ⁹ /l)	1.75±0.57	1.44±0.26	1.60±0.88	1.28±0.19	1.24±0.27	1.36±0.43	2.11±0.51	0.92±0.36
EO (10 ⁹ /l)	0.11±0.03	0.08±0.04	0.05±0.04	0.08±0.03	0.09±0.02	0.09±0.03	0.07±0.02	0.06±0.03
BASO(10 ⁹ /l)	0.04±0.01	0.05±0.01	0.04±0.03	0.05±0.02	0.04±0.02	0.05±0.02	0.06±0.02	0.02±0.02
LYM (%)	76.82±3.80	80.20±2.29	72.60±10.36	78.12±4.93	81.10±4.49	80.78±4.28	78.56±3.02	85.14±2.92
MONO (%)	6.08±1.10	4.84±0.53	5.76±1.13	6.64±2.21	5.30±1.27	4.86±1.23	3.86±0.91	3.42±0.55
NEUT (%)	15.74±2.90	13.78±1.97	20.48±9.57	13.78±2.46	12.26±3.31	13.06±3.04	16.58±2.61	10.48±2.36
EO (%)	0.98±0.33	0.76±0.29	0.86±0.83	0.84±0.27	0.88±0.24	0.84±0.21	0.56±0.17	0.66±0.15
BASO (%)	0.38±0.13	0.42±0.08	0.30±0.21	0.62±0.24	0.46±0.19	0.46±0.18	0.44±0.05	0.30±0.19
RBC (10 ¹² /l)	5.95±0.78	5.82±0.21	5.10±1.83	5.81±0.22	5.65±0.17	5.45±0.38	5.81±0.30	5.31±0.30
HGB (g/l)	130.00±15.36	127.40±5.64	111.60±38.64	130.20±4.60	125.20±1.10	119.40±6.50	130.60±7.30	123.00±6.20
HCT	37.20±3.76	37.12±1.64	32.34±10.88	37.42±1.47	35.28±0.44	33.30±2.19	35.80±1.98	33.84±2.01
MCV (fl)	62.64±2.04	63.78±0.90	64.36±3.85	64.44±0.15	62.52±1.63	61.14±2.08	61.64±0.45	63.76±1.37
MCH (pg)	21.90±0.23	21.90±0.35	22.06±1.01	22.40±0.25	22.26±0.68	21.98±0.89	22.50±0.25	23.18±0.67
MCHC (g/l)	349.60±7.77	343.20±2.59	342.40±8.91	348.00±3.39	356.20±3.56	359.80±7.19	364.80±6.46	363.80±5.45
RDW-SD(%)	35.58±1.81	35.74±2.05	37.68±2.50	36.98±1.90	33.88±1.66	34.28±0.97	34.54±1.52	35.46±2.70
RDW-CV	13.92±0.83	13.70±0.83	14.32±1.02	13.94±0.66	12.88±0.54	13.18±0.36	13.26±0.50	13.12±0.88
PLT (10 ⁹ /l)	925.00±397.8	835.4±193.14	842.6±453.34	906.4±279.53	878.2±412.72	1094.8±72.53	1057.6±64.01	982.8±418.07
MPV (fl)	5.56±0.42	5.78±0.45	5.44±0.11	5.66±0.35	6.00±0.37	5.18±0.24	5.60±0.23	5.32±0.24
PCT	0.50±0.20	0.48±0.10	0.46±0.25	0.51±0.14	0.52±0.24	0.57±0.06	0.59±0.03	0.52±0.21
PDW (fl)	15.62±0.19	15.66±0.11	16.02±0.81	15.68±0.11	15.84±0.33	15.48±0.15	15.68±0.13	15.84±0.43
Day 7	Male (vehicle)	Male (20 mg/kg)	Male (60 mg/kg)	Male (180 mg/kg)	Female (vehicle)	Female (20 mg/kg)	Female (60 mg/kg)	Female (180 mg/kg)
WBC(10 ⁹ /l)	8.01±3.55	10.50±2.12	8.74±1.64	8.04±1.44	7.97±0.77	9.11±1.90	8.22±2.62	8.76±2.33
LYM (10 ⁹ /l)	6.69±3.29	8.87±1.80	7.15±2.28	6.68±1.35	6.49±1.14	7.76±1.72	6.85±2.23	7.61±1.97
MONO(10 ⁹ /l)	0.43±0.11	0.48±0.18	0.33±0.10	0.42±0.03	0.36±0.05	0.37±0.10	0.34±0.18	0.27±0.07

NEUT($10^9/l$)	0.80±0.20	1.06±0.21	1.19±1.03	0.82±0.19	0.97±0.47	0.84±0.28	0.91±0.32	0.78±0.36
EO ($10^9/l$)	0.07±0.03	0.08±0.04	0.05±0.02	0.07±0.01	0.10±0.01	0.10±0.02	0.07±0.03	0.06±0.02
BASO($10^9/l$)	0.03±0.01	0.03±0.01	0.03±0.02	0.04±0.01	0.05±0.01	0.04±0.01	0.04±0.02	0.04±0.01
LYM (%)	82.36±3.67	84.38±2.70	80.58±14.95	82.90±2.72	80.94±8.32	84.80±4.24	83.26±3.14	86.98±1.08
MONO (%)	5.72±1.22	4.50±0.94	3.65±0.57	5.34±1.04	4.60±0.82	4.16±0.87	4.24±1.65	3.24±0.96
NEUT (%)	10.68±3.01	10.18±2.09	14.88±14.79	10.28±2.14	12.58±7.55	9.36±3.08	11.08±1.32	8.64±1.71
EO (%)	0.84±0.25	0.68±0.30	0.55±0.21	0.94±0.30	1.30±0.16	1.20±0.45	0.96±0.28	0.66±0.25
BASO (%)	0.40±0.20	0.26±0.09	0.35±0.24	0.54±0.15	0.58±0.19	0.48±0.13	0.46±0.15	0.48±0.08
RBC($10^{12}/l$)	6.05±0.51	5.96±0.43	6.22±0.78	5.90±0.24	5.60±0.25	5.73±0.40	5.91±0.25	5.81±0.59
HGB (g/l)	134.60±13.50	133.00±10.07	136.75±12.12	132.60±6.62	130.00±5.15	131.20±9.12	132.20±5.36	134.60±12.60
HCT	38.64±3.72	38.30±2.71	39.63±2.95	37.60±1.78	35.18±1.81	35.62±2.81	36.88±1.80	37.36±3.35
MCV (fl)	63.88±1.41	64.26±1.30	64.00±3.58	63.72±1.30	62.80±1.19	62.16±2.07	62.36±0.50	64.44±2.28
MCH (pg)	22.24±0.55	22.32±0.19	22.05±0.89	22.48±0.41	23.20±0.41	22.90±0.58	22.38±0.37	23.20±0.85
MCHC(g/l)	348.20±4.60	347.00±5.70	345.00±8.29	352.40±5.41	369.20±4.92	368.20±8.04	358.60±5.86	360.00±5.43
RDW-SD(%)	38.38±3.41	37.34±1.86	37.15±1.87	37.34±0.88	35.96±0.65	36.06±1.23	36.88±1.49	36.58±3.41
RDW-CV	14.80±1.31	14.16±0.71	13.93±0.73	14.00±0.48	13.36±0.30	13.40±0.48	13.86±0.63	13.26±1.00
PLT($10^9/l$)	1169.8±196.4	1211±134.22	1360.75±113.9	1205.2±86.46	1217.2±239.3	1337.8±96.76	1078.6±461.3	1150±434.39
MPV (fl)	5.44±0.18	5.56±0.30	5.70±0.29	5.54±0.27	5.54±0.29	5.20±0.24	5.62±0.19	5.32±0.26
PCT	0.64±0.11	0.67±0.08	0.77±0.08	0.66±0.04	0.68±0.13	0.70±0.04	0.60±0.25	0.61±0.23
PDW (fl)	15.64±0.17	15.74±0.15	15.88±0.13	15.76±0.11	15.90±0.17	15.74±0.15	15.90±0.35	15.84±0.26
Day 14	Male (vehicle)	Male (20 mg/kg)	Male (60 mg/kg)	Male (180 mg/kg)	Female (vehicle)	Female (20 mg/kg)	Female (60 mg/kg)	Female (180 mg/kg)
WBC($10^9/l$)	8.43±2.34	10.40±6.51	11.04±1.83	10.97±2.37	7.68±1.60	10.47±2.88	7.99±0.83	8.45±1.85
LYM($10^9/l$)	6.67±1.65	8.21±4.84	8.97±2.92	8.57±2.30	6.25±1.35	8.78±2.66	6.26±1.10	7.09±1.83
MONO($10^9/l$)	0.47±0.10	0.51±0.36	0.58±0.29	0.61±0.09	0.46±0.17	0.48±0.14	0.49±0.14	0.33±0.10
NEUT($10^9/l$)	1.15±0.60	1.58±1.41	1.37±0.86	1.68±0.59	0.80±0.35	1.04±0.33	1.11±0.39	0.95±0.43
EO ($10^9/l$)	0.09±0.03	0.07±0.04	0.08±0.04	0.07±0.02	0.10±0.05	0.10±0.03	0.07±0.02	0.07±0.03
BASO($10^9/l$)	0.05±0.03	0.03±0.03	0.05±0.01	0.04±0.01	0.07±0.03	0.08±0.03	0.05±0.03	0.02±0.01
LYM (%)	79.56±2.48	80.78±5.12	79.48±15.90	77.66±6.17	81.40±4.44	83.54±3.83	78.00±8.27	83.40±6.49
MONO (%)	5.64±1.41	4.36±1.39	5.80±4.08	5.64±1.13	6.10±2.56	4.60±1.23	6.20±2.02	3.86±0.57
NEUT (%)	13.08±2.87	13.90±4.46	13.60±11.47	15.64±5.48	10.26±3.70	10.14±2.54	14.14±5.80	11.68±6.67
EO (%)	1.14±0.30	0.66±0.11	0.75±0.49	0.68±0.29	1.38±0.74	0.94±0.19	0.96±0.26	0.82±0.30

BASO (%)	0.58±0.26	0.30±0.12	0.38±0.13	0.38±0.13	0.86±0.24	0.78±0.47	0.70±0.51	0.24±0.09
RBC(10 ¹² /l)	6.01±0.68	6.09±0.75	6.43±0.37	6.60±0.26	6.12±0.22	6.54±0.20	6.28±0.30	5.62±1.31
HGB (g/l)	133.40±8.26	135.20±15.04	144.25±2.06	145.60±5.81	133.80±3.56	137.80±4.32	135.60±4.56	126.20±27.22
HCT	37.42±2.88	38.82±3.74	40.38±0.59	41.48±1.76	38.84±0.63	40.46±1.44	39.00±1.87	36.44±7.95
MCV (fl)	62.52±2.80	63.88±2.03	62.95±3.80	62.76±0.80	63.46±1.49	61.94±3.13	62.10±1.07	65.08±2.56
MCH (pg)	22.28±1.42	22.20±0.38	22.53±1.46	22.06±0.39	21.88±0.61	21.10±0.92	21.60±0.41	22.52±1.24
MCHC(g/l)	356.00±7.31	347.60±5.59	357.50±2.65	351.20±2.95	344.80±5.07	341.00±4.42	348.00±6.20	346.00±5.57
RDW-SD(%)	36.76±2.34	36.34±0.38	38.65±2.60	33.76±2.16	38.64±1.63	36.92±0.63	36.92±2.27	35.82±3.00
RDW-CV	14.36±0.81	13.92±0.36	14.93±1.98	13.36±0.82	14.46±0.57	14.18±0.60	14.24±0.64	13.32±0.83
PLT(10 ⁹ /l)	1008.8±482.4	919.6±520.16	1152.0±78.64	1074.0±210.5	857.6±388.18	839.8±430.25	961.0±199.59	1008.6±407.8
MPV (fl)	6.48±0.44	6.24±0.38	6.48±0.22	6.46±0.24	6.44±0.25	6.24±0.19	6.46±0.11	6.24±0.28
PCT	0.64±0.29	0.58±0.32	0.75±0.07	0.69±0.11	0.55±0.23	0.52±0.27	0.62±0.12	0.62±0.25
PDW (fl)	15.90±0.79	16.42±2.01	15.53±0.05	15.40±0.12	16.02±0.89	15.94±0.77	15.58±0.08	15.48±0.30
Day 21	Male (vehicle)	Male (20 mg/kg)	Male (60 mg/kg)	Male (180 mg/kg)	Female (vehicle)	Female (20 mg/kg)	Female (60 mg/kg)	Female (180 mg/kg)
WBC(10 ⁹ /l)	9.84±5.63	10.72±2.78	9.34±3.60	7.77±0.87	11.58±2.17	11.02±0.77	12.27±2.52	7.26±4.12
LYM (10 ⁹ /l)	7.87±4.98	8.59±2.50	6.98±2.49	5.50±0.83	9.79±1.68	9.34±0.51	10.34±1.58	5.94±3.29
MONO(10 ⁹ /l)	0.54±0.29	0.58±0.33	0.64±0.81	0.55±0.19	0.40±0.26	0.40±0.03	0.34±0.15	0.39±0.30
NEUT(10 ⁹ /l)	1.23±0.95	1.36±0.19	1.57±1.16	1.55±0.45	1.22±0.35	1.13±0.21	1.45±0.80	0.78±0.50
EO (10 ⁹ /l)	0.12±0.08	0.10±0.03	0.10±0.06	0.12±0.09	0.13±0.04	0.10±0.03	0.08±0.02	0.08±0.06
BASO(10 ⁹ /l)	0.08±0.06	0.08±0.05	0.05±0.04	0.05±0.02	0.04±0.01	0.05±0.02	0.06±0.01	0.07±0.06
LYM (%)	79.30±8.75	79.58±4.88	77.68±15.16	70.80±7.55	84.70±2.55	84.90±1.44	84.90±4.33	81.04±5.08
MONO (%)	5.58±1.84	5.18±1.66	6.22±6.99	7.16±2.79	3.23±1.60	3.57±0.23	2.67±0.75	4.84±1.76
NEUT (%)	12.96±6.65	13.42±4.59	14.56±8.50	19.86±5.24	10.57±2.66	10.17±1.23	11.33±3.95	12.14±4.60
EO (%)	1.44±0.61	1.06±0.31	1.04±0.44	1.46±0.97	1.13±0.21	0.90±0.20	0.63±0.15	1.26±0.46
BASO (%)	0.72±0.40	0.76±0.29	0.50±0.29	0.72±0.23	0.37±0.06	0.47±0.15	0.47±0.15	0.72±0.54
RBC(10 ¹² /l)	7.19±0.59	7.12±0.52	6.47±0.70	6.87±0.31	6.37±0.66	6.21±0.55	6.51±0.19	5.44±1.01
HGB (g/l)	152.60±15.68	150.20±10.71	137.20±11.82	146.20±6.10	140.00±10.82	137.00±7.81	142.33±2.08	123.20±21.16
HCT	42.44±3.74	42.40±2.65	39.06±3.66	41.42±1.52	39.23±4.05	37.97±1.97	39.23±0.57	35.06±5.46
MCV (fl)	59.00±1.70	59.58±1.09	60.60±3.60	60.36±1.44	61.63±2.23	61.33±3.74	60.27±1.76	64.86±2.62
MCH (pg)	21.16±0.71	21.12±0.53	21.32±1.33	21.30±0.73	22.07±0.78	22.13±1.32	21.87±0.42	22.70±1.05
MCHC (g/l)	358.60±7.30	354.40±5.59	351.80±4.09	352.80±3.70	358.33±9.29	361.00±2.00	362.67±7.64	349.80±10.78

RDW-SD(%)	34.82±1.99	35.02±0.69	36.16±3.12	33.26±0.74	39.17±2.43	39.80±1.56	37.97±2.05	40.58±1.32
RDW-CV	14.44±0.64	14.42±0.50	14.88±2.22	13.58±0.56	14.87±0.61	15.20±0.72	15.00±0.44	14.86±0.58
PLT(10 ⁹ /l)	667.20±369.8	689.2±195.64	660.60±503.83	859.8±422.04	895.0±490.27	1,075.0±45.03	698.3±515.99	633.8±422.61
MPV (fl)	5.92±0.22	6.30±0.28	6.30±0.21	5.92±0.34	6.10±0.61	5.70±0.10	5.93±0.42	5.50±0.32
PCT	0.40±0.23	0.43±0.11	0.41±0.30	0.50±0.24	0.53±0.26	0.61±0.02	0.40±0.29	0.35±0.24
PDW (fl)	15.88±0.28	15.96±0.31	16.00±0.48	15.74±0.44	16.27±0.81	15.67±0.06	16.47±1.42	16.14±0.73
Day 28	Male (vehicle)	Male (20 mg/kg)	Male (60 mg/kg)	Male (180 mg/kg)	Female (vehicle)	Female (20 mg/kg)	Female (60 mg/kg)	Female (180 mg/kg)
WBC (10 ⁹ /l)	10.47±1.21	10.53±1.04	11.23±2.97	12.17±3.15	10.90±2.61	13.05±3.44	10.98±1.85	10.13±0.87
LYM (10 ⁹ /l)	8.34±0.76	8.72±1.01	8.79±2.60	9.07±2.34	9.12±2.26	11.12±3.65	9.55±1.78	8.49±0.65
MONO(10 ⁹ /l)	0.58±0.21	0.42±0.12	0.63±0.18	1.02±0.49	0.32±0.06	0.51±0.16	0.30±0.05	0.38±0.08
NEUT(10 ⁹ /l)	1.42±0.61	1.26±0.06	1.64±0.85	1.88±0.49	1.32±0.44	1.26±0.41	1.01±0.12	1.12±0.15
EO (10 ⁹ /l)	0.09±0.03	0.11±0.04	0.12±0.04	0.12±0.04	0.11±0.02	0.12±0.05	0.09±0.02	0.10±0.06
BASO(10 ⁹ /l)	0.04±0.01	0.03±0.01	0.05±0.03	0.08±0.03	0.05±0.02	0.04±0.02	0.03±0.02	0.04±0.03
LYM (%)	79.95±5.59	82.70±1.85	78.40±8.71	74.62±3.58	83.44±3.65	84.04±6.71	86.80±2.00	83.90±1.66
MONO (%)	5.48±1.53	3.90±0.85	5.60±1.19	8.24±2.26	3.00±0.58	4.16±1.58	2.78±0.56	3.72±0.66
NEUT (%)	13.30±4.24	12.03±1.89	14.43±7.12	15.54±2.27	12.14±3.11	10.62±5.31	9.38±1.65	11.02±0.89
EO (%)	0.88±0.19	1.03±0.25	1.15±0.42	1.00±0.29	0.98±0.26	0.86±0.22	0.85±0.10	0.96±0.53
BASO (%)	0.40±0.08	0.33±0.12	0.43±0.17	0.60±0.10	0.44±0.22	0.32±0.15	0.20±0.16	0.40±0.19
RBC (10 ¹² /l)	6.77±0.17	6.68±0.36	6.61±0.53	6.66±0.93	6.32±0.25	6.17±0.53	6.51±0.64	5.85±0.54
HGB (g/l)	144.75±3.77	145.00±7.21	143.25±8.30	142.80±13.05	138.60±2.70	133.40±6.31	136.50±13.82	131.80±6.65
HCT	38.40±1.09	38.13±1.85	37.65±1.66	38.28±4.41	37.70±1.28	35.70±2.23	37.45±3.46	35.44±2.12
MCV (fl)	56.78±1.55	57.03±0.86	57.15±3.23	57.64±1.98	59.68±2.01	57.96±1.91	57.58±0.72	60.74±2.74
MCH (pg)	21.40±0.54	21.70±0.56	21.70±1.22	21.58±1.31	21.96±0.57	21.68±1.08	20.95±0.57	22.62±1.28
MCHC (g/l)	377.00±5.72	380.00±4.58	379.75±6.13	374.20±16.42	367.60±5.46	374.20±8.84	364.25±6.70	372.40±5.98
RDW-SD(%)	33.73±1.69	33.93±0.57	34.25±3.43	33.48±1.46	35.94±1.74	34.00±1.44	32.68±1.43	34.52±1.47
RDW-CV	14.10±0.49	14.10±0.30	14.33±2.02	13.82±0.74	14.10±0.42	13.66±0.51	13.35±0.48	13.34±0.25
PLT (10 ⁹ /l)	911.75±318.3	963.0±148.62	822.50±505.56	835.0±288.04	999.20±273.99	1033.2±102.84	866.00±357.09	988.40±314.22
MPV (fl)	5.93±0.39	5.97±0.31	6.28±0.52	5.76±0.38	5.82±0.44	5.98±0.27	5.70±0.18	5.84±0.29
PCT	0.53±0.16	0.57±0.06	0.50±0.30	0.48±0.17	0.57±0.13	0.62±0.05	0.50±0.21	0.57±0.16
PDW (fl)	15.83±0.26	15.77±0.15	16.78±2.08	15.84±0.23	15.66±0.37	15.88±0.08	15.48±0.19	15.70±0.41

Table S3. The biochemistry index of rats in subacute toxicity.

Day 0	Male (vehicle)	Male (20 mg/kg)	Male (60 mg/kg)	Male (180 mg/kg)	Female (vehicle)	Female (20 mg/kg)	Female (60 mg/kg)	Female (180 mg/kg)
ALT(U/l)	55.60±6.80	49.40±8.73	45.80±4.97	51.20±12.07	48.80±26.87	72.00±53.25	44.20±5.54	51.80±5.07
AST(U/l)	138.60±44.96	132.80±22.10	120.6±18.82	96.40±54.45	152.40±34.20	126.20±14.01	133.6±35.42	113.4±22.81
TBA(μM)	77.78±51.81	73.62±31.22	40.40±23.72	67.16±31.68	54.08±31.26	41.60±14.37	54.16±37.08	49.48±27.57
CHO(mM)	2.35±0.27	2.05±0.16	2.09±0.21	1.91±0.79	2.08±0.27	2.00±0.12	2.09±0.35	2.16±0.23
HDL(mM)	0.96±0.09	0.77±0.08	0.79±0.08	0.75±0.32	0.85±0.14	0.74±0.04	0.76±0.11	0.79±0.08
LDL(mM)	0.37±0.08	0.33±0.03	0.30±0.03	0.23±0.10	0.29±0.04	0.26±0.04	0.27±0.04	0.27±0.04
TG(mM)	0.73±0.19	0.62±0.32	0.87±0.35	0.88±0.52	0.66±0.12	0.64±0.15	0.71±0.36	1.02±0.11
ALB(g/l)	27.72±1.76	23.72±3.51	25.14±1.69	25.70±3.06	28.96±2.22	27.46±2.64	26.30±2.68	26.28±1.23
GLU(μM)	6.36±0.55	5.47±0.89	5.45±0.30	4.65±2.31	6.17±0.69	5.49±0.35	5.61±0.72	5.58±0.36
Urea(μM)	5.17±1.06	4.03±0.48	3.78±0.40	3.44±1.68	5.59±0.31	4.81±0.56	5.10±0.57	5.25±0.57
Cre(μM)	46.20±2.59	41.20±3.49	41.20±2.86	32.80±18.43	48.80±4.60	43.80±3.42	46.40±3.97	45.40±2.19
TP(g/l)	53.56±3.32	45.28±5.90	46.61±3.16	39.89±20.81	55.02±3.99	53.15±4.37	50.44±5.03	50.96±3.67
TBiLi(μM)	1.24±0.60	1.40±0.23	1.00±0.42	0.50±0.91	0.82±0.71	1.06±0.53	0.64±0.59	0.60±0.45
D.BIL(μM)	-0.76±0.68	-0.24±0.32	-0.70±0.48	-0.62±1.62	-1.18±0.87	-1.06±0.81	-1.08±0.45	-0.86±0.63
UA(μM)	115.06±18.31	114.56±11.99	132.22±17.93	122.44±40.46	119.54±31.48	102.26±23.14	106.6±32.37	91.58±17.61
Day 7	Male (vehicle)	Male (20 mg/kg)	Male (60 mg/kg)	Male (180 mg/kg)	Female (vehicle)	Female (20 mg/kg)	Female (60 mg/kg)	Female (180 mg/kg)
ALT(U/l)	50.40±3.29	39.40±22.94	47.20±10.92	55.00±14.20	47.40±8.99	43.80±5.81	46.60±4.34	51.20±7.05
AST(U/l)	126.20±24.73	63.80±35.25	94.00±12.88	97.40±28.22	139.40±72.30	80.80±8.53	86.80±11.08	77.60±15.01
TBA(μM)	72.60±32.24	60.96±42.01	40.70±33.92	53.26±46.33	51.42±41.85	32.70±27.38	68.24±24.43	49.98±33.61
CHO(mM)	1.99±0.14	1.55±0.86	1.86±0.36	1.76±0.16	1.90±0.22	2.06±0.20	1.95±0.15	1.81±0.22

HDL(mM)	0.78±0.05	0.63±0.36	0.76±0.18	0.79±0.06	0.73±0.10	0.78±0.06	0.78±0.07	0.73±0.08
LDL(mM)	0.30±0.05	0.20±0.12	0.24±0.04	0.25±0.04	0.23±0.04	0.24±0.02	0.22±0.04	0.21±0.02
TG(mM)	1.16±0.15	1.00±0.60	1.21±0.52	1.32±0.41	1.13±0.29	1.14±0.28	0.85±0.19	0.99±0.32
ALB(g/l)	22.34±1.11	19.22±10.79	23.90±2.55	23.90±1.33	25.46±2.04	26.20±1.05	26.96±2.09	25.48±1.57
GLU(μM)	5.17±0.28	4.23±2.41	6.24±1.25	5.23±0.68	5.42±0.31	4.97±0.29	5.36±0.32	5.08±0.23
Urea(μM)	5.00±0.40	4.17±2.34	5.99±1.14	5.01±0.27	5.12±0.29	5.42±0.70	5.96±0.49	6.05±0.52
Cre(μM)	49.00±3.54	39.00±20.24	53.20±4.60	51.60±2.70	54.00±2.35	53.40±1.34	55.00±3.94	54.80±1.79
TP(g/l)	48.95±2.82	41.63±22.69	52.47±6.59	51.66±3.02	53.30±5.19	54.62±1.87	57.11±4.81	53.15±2.83
TBiLi(μM)	0.96±0.44	0.72±0.37	0.74±0.65	0.78±0.38	0.64±0.45	0.76±0.51	1.04±0.53	0.64±0.44
D.BIL(μM)	-0.90±0.29	-0.54±0.80	-1.04±0.91	-1.14±0.38	-1.98±0.90	-1.26±0.52	-1.64±0.40	-1.34±0.52
UA(μM)	110.16±11.41	81.66±55.72	127.86±22.69	106.98±15.55	128.66±36.68	115.68±15.53	121.48±32.04	112.48±20.5
Day 14	Male (vehicle)	Male (20 mg/kg)	Male (60 mg/kg)	Male (180 mg/kg)	Female (vehicle)	Female (20 mg/kg)	Female (60 mg/kg)	Female (180 mg/kg)
ALT(U/l)	46.00±2.00	47.60±8.79	41.40±4.98	47.40±5.03	46.80±3.70	48.40±8.59	44.20±3.35	61.40±46.04
AST(U/l)	134.00±26.47	77.20±10.18	82.60±27.12	82.40±12.66	151.60±48.19	107.00±33.56	87.00±11.90	123.6±115.9
TBA(μM)	66.02±29.68	95.90±26.23	35.78±12.69	64.54±35.85	47.68±22.84	72.58±54.00	83.20±24.10	29.08±26.98
CHO(mM)	1.87±0.26	1.73±0.17	1.63±0.24	1.62±0.13	1.92±0.17	2.07±0.21	1.99±0.33	1.84±0.18
HDL(mM)	0.73±0.10	0.70±0.06	0.65±0.07	0.66±0.05	0.72±0.05	0.78±0.06	0.72±0.06	0.68±0.08
LDL(mM)	0.21±0.07	0.23±0.03	0.18±0.04	0.22±0.04	0.27±0.04	0.24±0.04	0.21±0.04	0.20±0.04
TG(mM)	1.11±0.23	1.02±0.17	1.15±0.27	1.21±0.24	0.81±0.11	0.97±0.38	0.75±0.24	0.91±0.23
ALB(g/l)	22.68±0.41	21.64±1.11	20.64±1.13	21.48±1.52	25.68±1.55	25.46±1.05	24.64±1.50	23.74±1.49
GLU(μM)	4.98±0.29	4.93±0.39	5.57±0.59	5.18±0.51	5.18±0.81	5.95±0.96	5.09±0.28	5.33±0.36
Urea(μM)	4.61±0.16	4.49±0.28	4.60±0.48	3.96±0.28	5.34±0.34	5.30±0.85	5.01±0.43	4.56±0.42

Cre(μ M)	51.80 \pm 2.77	49.60 \pm 3.71	49.00 \pm 3.24	48.60 \pm 1.82	60.60 \pm 3.78	57.20 \pm 2.59	56.60 \pm 3.85	52.20 \pm 4.15
TP(g/l)	49.84 \pm 1.23	48.04 \pm 1.85	45.46 \pm 2.40	48.51 \pm 0.99	54.94 \pm 2.72	54.16 \pm 1.71	54.19 \pm 2.13	50.79 \pm 3.03
TBiLi(μ M)	0.96 \pm 0.59	1.42 \pm 0.19	0.76 \pm 0.43	0.80 \pm 0.35	0.82 \pm 0.44	1.10 \pm 0.85	0.98 \pm 0.50	1.00 \pm 0.51
D.BIL(μ M)	-1.06 \pm 0.61	-0.64 \pm 0.36	-2.96 \pm 3.79	-1.12 \pm 0.36	-1.56 \pm 0.59	-1.92 \pm 1.76	-1.66 \pm 1.09	-1.10 \pm 0.33
UA(μ M)	101.14 \pm 19.79	85.04 \pm 17.25	90.58 \pm 27.22	100.32 \pm 22.71	127.54 \pm 27.52	90.50 \pm 28.05	80.68 \pm 18.38	76.66 \pm 13.71
Day21	Male (vehicle)	Male (20 mg/kg)	Male (60 mg/kg)	Male (180 mg/kg)	Female (vehicle)	Female (20 mg/kg)	Female (60 mg/kg)	Female (180 mg/kg)
ALT(U/l)	49.20 \pm 2.28	45.20 \pm 6.83	46.20 \pm 7.01	55.00 \pm 6.40	40.00 \pm 3.87	45.00 \pm 2.45	40.80 \pm 2.17	43.00 \pm 6.32
AST(U/l)	121.20 \pm 28.47	74.00 \pm 6.52	88.60 \pm 18.34	89.80 \pm 9.96	134.40 \pm 11.59	75.00 \pm 4.18	66.40 \pm 7.73	68.60 \pm 6.23
TBA(μ M)	79.64 \pm 44.85	62.26 \pm 42.61	38.78 \pm 5.78	57.64 \pm 42.23	54.36 \pm 32.89	99.72 \pm 66.86	61.84 \pm 20.83	58.68 \pm 37.25
CHO(mM)	1.81 \pm 0.17	1.66 \pm 0.22	1.56 \pm 0.32	1.73 \pm 0.34	1.74 \pm 0.14	2.32 \pm 0.23	1.75 \pm 0.25	1.90 \pm 0.27
HDL(mM)	0.77 \pm 0.09	0.75 \pm 0.05	0.70 \pm 0.12	0.73 \pm 0.09	0.69 \pm 0.07	0.79 \pm 0.07	0.68 \pm 0.09	0.72 \pm 0.07
LDL(mM)	0.22 \pm 0.05	0.19 \pm 0.06	0.16 \pm 0.05	0.19 \pm 0.04	0.19 \pm 0.01	0.24 \pm 0.05	0.20 \pm 0.06	0.18 \pm 0.02
TG(mM)	1.47 \pm 0.23	1.21 \pm 0.23	1.43 \pm 0.31	1.08 \pm 0.38	0.79 \pm 0.27	1.07 \pm 0.50	0.67 \pm 0.16	0.84 \pm 0.33
ALB(g/l)	27.04 \pm 2.93	24.78 \pm 1.93	25.80 \pm 3.04	25.96 \pm 2.65	25.94 \pm 1.73	27.34 \pm 1.49	26.02 \pm 1.08	26.02 \pm 0.90
GLU(μ M)	5.22 \pm 0.29	5.40 \pm 0.38	5.42 \pm 0.32	6.17 \pm 1.01	5.11 \pm 0.37	5.95 \pm 0.47	5.65 \pm 0.37	5.65 \pm 0.63
Urea(μ M)	5.28 \pm 0.56	5.10 \pm 0.39	5.29 \pm 0.87	5.08 \pm 0.38	5.30 \pm 0.41	4.86 \pm 0.10	5.79 \pm 0.34	5.27 \pm 0.17
Cre(μ M)	52.00 \pm 2.24	53.20 \pm 3.19	51.60 \pm 4.56	53.60 \pm 3.78	57.00 \pm 2.92	57.00 \pm 1.58	55.40 \pm 1.67	56.00 \pm 2.00
TP(g/l)	61.13 \pm 4.30	56.60 \pm 3.36	56.40 \pm 6.81	57.85 \pm 4.10	57.35 \pm 2.33	61.75 \pm 2.15	56.85 \pm 1.35	57.49 \pm 1.89
TBiLi(μ M)	0.98 \pm 0.35	1.20 \pm 0.24	0.62 \pm 0.55	0.70 \pm 0.39	0.70 \pm 0.51	1.30 \pm 0.91	1.08 \pm 0.76	1.20 \pm 0.85
D.BIL(μ M)	-0.86 \pm 0.58	-0.64 \pm 0.32	-0.96 \pm 0.36	-1.04 \pm 0.55	-1.28 \pm 0.15	-2.24 \pm 2.69	-1.02 \pm 0.43	-0.62 \pm 0.37
UA(μ M)	108.76 \pm 24.34	87.82 \pm 16.46	111.82 \pm 8.90	121.02 \pm 29.63	121.64 \pm 28.86	110.58 \pm 5.41	89.08 \pm 9.93	78.56 \pm 10.64
Day 28	Male (vehicle)	Male (20 mg/kg)	Male (60 mg/kg)	Male (180 mg/kg)	Female (vehicle)	Female (20 mg/kg)	Female (60 mg/kg)	Female (180 mg/kg)

ALT(U/l)	38.60±11.67	35.20±10.62	35.00±12.59	46.00±16.31	38.20±10.45	37.60±12.58	35.20±10.26	31.20±10.08
AST(U/l)	116.60±36.05	80.00±24.00	60.60±14.36	85.00±13.36	112.60±43.38	65.20±13.66	72.20±16.54	75.80±41.98
TBA(μM)	36.88±18.06	87.42±82.21	14.86±11.61	31.42±13.06	27.36±17.89	53.86±39.10	37.70±21.99	43.96±38.95
CHO(mM)	1.21±0.36	1.12±0.50	1.07±0.52	1.33±0.31	1.56±0.34	1.73±0.38	1.51±0.35	1.51±0.28
HDL(mM)	0.52±0.16	0.49±0.21	0.47±0.24	0.58±0.15	0.62±0.15	0.67±0.16	0.61±0.15	0.58±0.13
LDL(mM)	0.12±0.07	0.09±0.07	0.08±0.06	0.14±0.05	0.12±0.04	0.11±0.04	0.11±0.05	0.10±0.04
TG(mM)	1.15±0.55	0.95±0.53	1.01±0.88	0.99±0.65	0.79±0.43	0.85±0.45	0.71±0.27	0.53±0.45
ALB(g/l)	20.62±6.94	20.32±7.62	18.34±7.69	21.96±5.80	25.56±6.18	24.88±5.58	25.22±7.15	22.04±4.97
GLU(μM)	8.09±4.22	6.78±2.26	7.15±2.16	9.55±4.82	8.16±4.75	6.48±2.07	7.96±4.15	6.33±2.40
Urea(μM)	5.02±0.99	4.69±1.06	4.55±1.03	4.94±0.38	5.46±1.24	4.72±0.92	5.02±1.20	4.58±1.29
Cre(μM)	45.40±10.09	41.80±9.76	42.80±8.47	55.60±5.50	56.80±2.68	54.20±6.22	54.00±6.44	51.80±4.76
TP(g/l)	44.50±16.52	43.47±16.47	40.13±19.07	50.89±11.85	52.56±12.17	53.16±12.64	51.93±15.96	47.13±9.95
TBiLi(μM)	0.90±0.21	0.96±0.51	0.50±0.46	0.76±0.17	0.60±0.21	0.84±0.67	0.86±0.86	0.88±0.76
D.BIL(μM)	-0.74±0.26	-0.64±0.41	-0.66±0.38	-0.88±0.36	-1.08±0.40	-0.92±0.39	-0.96±0.51	-0.62±0.65
UA(μM)	116.00±33.51	119.44±24.19	87.66±12.18	98.22±49.06	146.50±17.53	105.90±23.79	107.42±23.68	86.42±16.23

Table S4. Metabolic Stability of Z583 and control compound (verapamil) in Different Species of Hepatocytes

Compound	Species	In vitro $T_{1/2}$ (min)	In vitro Cl_{int} ($\mu\text{l}/\text{min}, 10^6$ cells)	Scale-up Cl_{int} (m/min/kg)
Verapamil	Human	24.78	55.94	142.33
	Mouse	24.16	57.37	394.68
	Dog	14.58	95.07	1123.01
Z583	Human	61.25	22.63	57.57
	Mouse	108.10	12.82	88.21
	Dog	26.66	51.98	614.05

Note: If $Cl_{int} < 0$, then $T_{1/2}$ and Cl_{int} were reported as “ ∞ ” and “0.00”, respectively.

Table S5. The stability results of Z583 and control compounds (propranolol and mevinolin) in human, mouse and dog whole blood.

Compound	Species	$T_{1/2}$ (min)	Remaining Percentages(%)				
			0 min	15 min	30 min	60 min	120 min
Propranolol	Human	32.05	100.00	80.18	58.82	31.89	7.73
Propranolol	Mouse	57.03	100.00	82.78	72.25	46.46	23.57
Mevinolin	Dog	162.44	100.00	95.23	92.27	86.05	59.60
Z583	Human	190.08	100.00	94.49	90.44	82.17	64.34
Z583	Mouse	70.04	100.00	85.28	71.17	43.85	31.53
Z583	Dog	105.44	100.00	89.02	80.49	64.63	45.33

Table S6. Recombinant human and mouse cytokines involved in the experiments.

Cytokines	Identifier	Supplier
Recombinant Human IL-2	202-IL-010	R&D
Recombinant Human IL-4	204-IL-010	R&D
Recombinant Human IL-6	206-IL-010	R&D
Recombinant Human IL-7	207-IL-010	R&D
Recombinant Human IL-9	209-ILB-010	R&D
Recombinant Human IL-10	217-IL-010	R&D
Recombinant Human IL-13	213-ILB-010	R&D
Recombinant Human IL-15	247-ILB-010	R&D
Recombinant Human IL-21	8879-IL-010	R&D
Recombinant Human EPO	286-EP-250	R&D
Recombinant Human GM-CSF	300-03	R&D
Recombinant Human IFN- γ	300-02	R&D
Recombinant Mouse IL-2	212-12	Peprotech
Recombinant Mouse IL-4	214-14	Peprotech
Recombinant Mouse IL-6	210-16	Peprotech
Recombinant Mouse TNF- α	718004	Peprotech
Recombinant Mouse IL-12	210-12	Peprotech
Recombinant Mouse IL-13	571104	Peprotech
Recombinant Mouse IL-15	210-15	Peprotech
Recombinant Mouse IL-1 β	211-11B	Peprotech
Recombinant Mouse IL-23	589002	Peprotech
Recombinant Mouse IFN- γ	315-05	Peprotech
Recombinant Mouse GM-CSF	315-03	Peprotech
Recombinant Human TGF- β 1	100-21	Peprotech