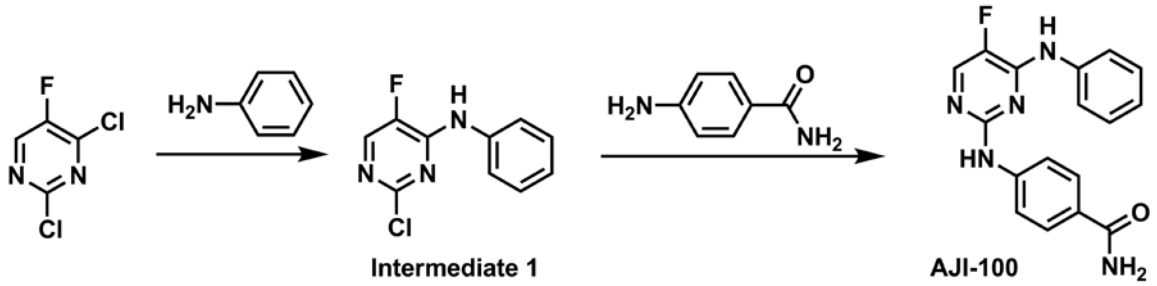
Supplemental Fig. 1. Synthesis of AJI-214 and AJI-100



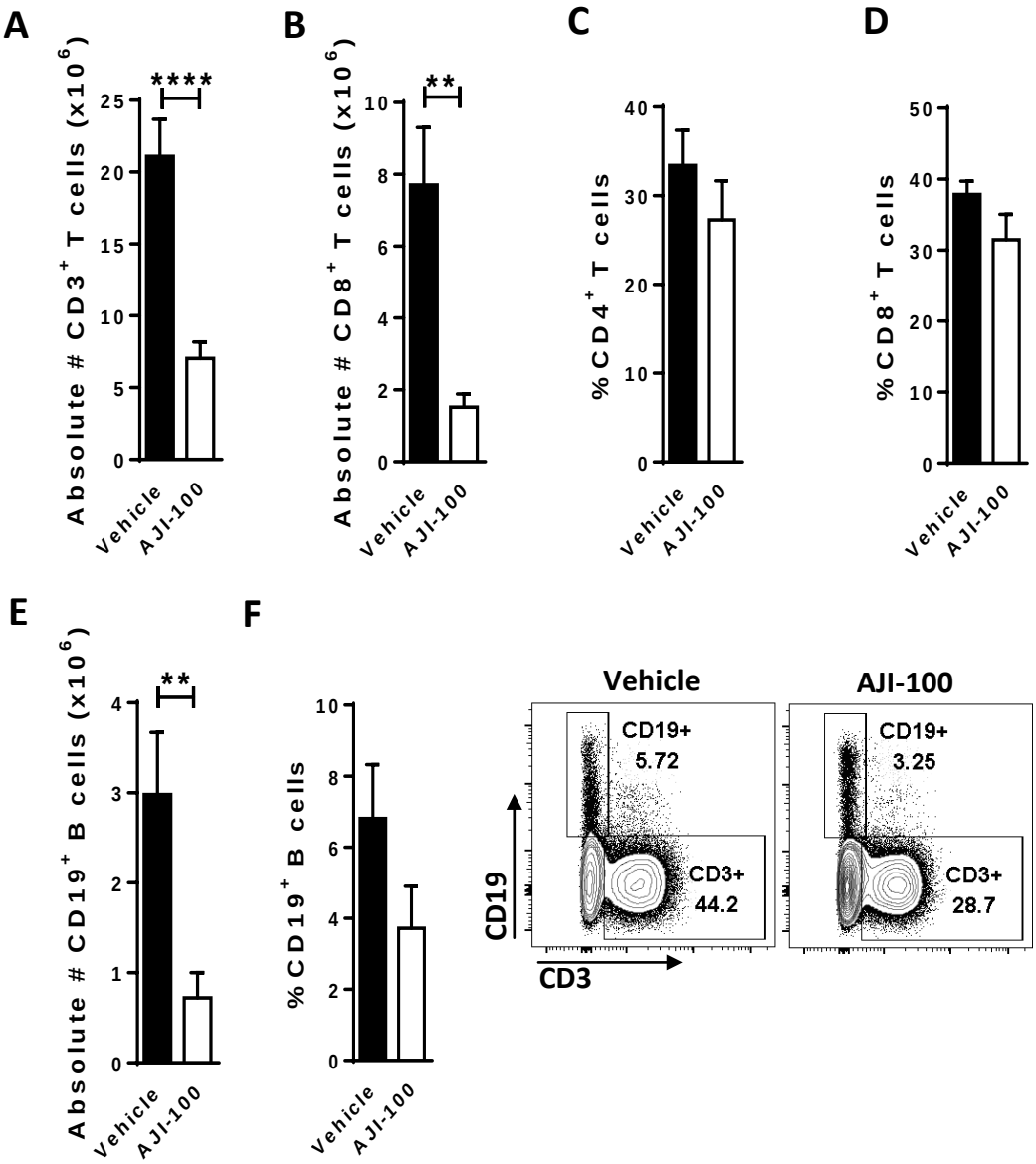
The bisanilinopyrimidine **AJI-214** was prepared using the method we have previously reported*(30)*. The bisanilinopyrimidine **AJI-100** was prepared using the two step route we have used to prepare other 2,4-dianilinopyrimidines. Reaction of 2,4-dichloro-5-fluoropyrimidine with aniline provided the **intermediate 1**. **Intermediate 1** was reacted further with 4-aminobenzamide to give the required **AJI-100**, with HPLC purity > 99%.

2-Chloro-5-fluoro-*N*-phenylpyrimidin-4-amine (intermediate 1): To a solution of 5-fluoro-2,4-dichloropyrimidine (2.00 g, 11.98 mmol) and diethylisopropylamine (2.50 mL, 14.37 mmol) in isopropanol (12 mL) was added aniline (1.09 mL, 11.98 mmol). The mixture was stirred at reflux for 1 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in dichloromethane (20 mL) and washed with water (10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The resulting solid was triturated using EtOAc/hexanes to give the title compound as a white solid (1.35 g, 50%). Mp: 135-136 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.99 (s, 1H, disappeared on D_2O shake), 8.30 (d, J = 3.5 Hz, 1H), 7.66 (d, J = 8.0 Hz, 2H), 7.37 (t, J = 8.0 Hz, 2H), 7.13 (t, J = 8.0 Hz, 1H). ^{19}F NMR (376 MHz, DMSO-d_6): δ -153.7 (s). HPLC–MS (ESI+): *m/z* 226.1 [40%, $(M^{37}Cl+H)^+$], 224.1 [100%, $(M^{35}Cl+H)^+$].

5-Fluoro-N^4-phenyl-N^2-[4-(4-carboxamide)phenyl]pyrimidine-2,4-diamine (AJI-100): A mixture of **intermediate 1** (1.00 g, 4.47 mmol), 4-aminobenzamide (0.609 g, 4.47 mmol), and methanol (4.5 mL) was heated at 100 °C for 14 h. The reaction mixture was cooled to room temperature and the precipitate filtered and washed with MeOH (2 × 10 mL). The resulting solid was sonicated in saturated sodium bicarbonate solution (10 mL) for 2 min, then filtered, washed with water (3 × 20 mL), MeOH (2 × 10 mL), and dried to give **AJI-100** as a white solid (1.07 g, 74%). Mp: 248-249 °C. HPLC: 99.9% [t_R = 10.9 min, 50% MeOH, 50% water (with 0.1% TFA), 20 min]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.51 (s, 1H, disappeared on D_2O shake), 9.44 (s, 1H, disappeared on D_2O shake), 8.14 (d, J = 3.7 Hz, 1H), 7.77 (brs, 1H, disappeared on D_2O shake), 7.76 (d, J = 7.8 Hz, 2H), 7.72 (s, 4H), 7.36 (t, J = 7.8 Hz, 2H), 7.12 (brs, 1H, disappeared on D_2O shake), 7.10 (t, J = 7.8 Hz, 1H). ^{19}F NMR (376 MHz, DMSO-d_6): δ -163.2 (s). HPLC–MS (ESI+): *m/z* 324.2 [100%, $(M+H)^+$]. LC–MS (ESI+): 992.3 [20%, $(3M+Na)^+$], 669.2 [50%, $(2M+Na)^+$], 346.1 [30%, $(M+Na)^+$], 324.1 [100%, $(M+H)^+$]. HRMS (ESI+): *m/z* calcd for $C_{17}H_{14}FN_5O$ $(M+H)^+$ 324.1255, found 324.1262.

Kinases	AJI-100 (IC50 in nM)	Staurosporine (IC50 in nM)
AMPK(A1/B2/G1)	0.4	0.062
Aurora A	**12.7**	1.7
JAK2	**18.5**	0.16
ARK5/NUAK1	21.9	1.1
Aurora B	40	8.33
MLK1/MAP3K9	50.1	1.32
MLK3/MAP3K11	81.6	4.45
MINK/MINK1	134	0.53
DYRK2	168	164
KDR/VEGFR2	197	10.3
MELK	215	0.94
YES/YES1	223	1.82
c-Src	270	2.55
CLK2	296	8.37
PIM3	305	0.15
TRKA	397	3.29
IGF1R	408	33.3
TAK1	411	50.4
MST2/STK3	514	5.53
DYRK3	555	39.7
MLCK/MYLK	564	56.6
GLK/MAP4K3	575	0.13

Supplemental Fig. 2. AJI-100 kinase target screen. The selectivity of AJI-100 (250nM) was first tested against a panel of 140 kinase targets at the International Centre for Kinase Profiling (ICKP, http://www.kinase-screen.mrc.ac.uk/). The IC_{50} values for the top 22 targets were then measured (using the Reaction Biology Hotspot assay) for AJI-100 and compared against the nonselective control compound, staurosporine. This screen shows AJI-100 selectively inhibits Aurora kinase A and JAK2 (both bold and highlighted) at low nanomolar concentrations. The majority of off-target kinases suppressed by AJI-100 have IC_{50} values well above 100nM, further demonstrating the selectivity of AJI-100. (Note: the Aurora kinase A IC_{50} for alisertib is 1.2nM (Aurora A:B = 1:27)*(27,31)*, and the JAK2 IC_{50} for TG101348 is 3nM*(26)*. The Aurora kinase A and JAK2 IC_{50} values for AJI-214 are 5.7nM and 33.4nM, respectively)*(28)*.



Supplemental Fig. 3. Effects of AJI-100 on immune reconstitution. NSG mice received 30x10^6 human PBMCs and were then treated with AJI-100 (50mg/kg daily) or vehicle from day 0 to day +14. Recipient spleens were harvested and tissue-resident T cells were evaluated. **A-D)** AJI-100 significantly reduces the total number of T cells in the spleen, but the percentages of CD4$^+$ and CD8$^+$ subsets are similar to vehicle. **E,F)** AJI-100 significantly suppresses B cell engraftment, but the proportion of T cells to B cells in the spleen is similar to vehicle. Pooled data from at least 2 independent experiments. n=6-14 mice per each group. Mann-Whitney. **P=.001-.01, ****P<.0001.