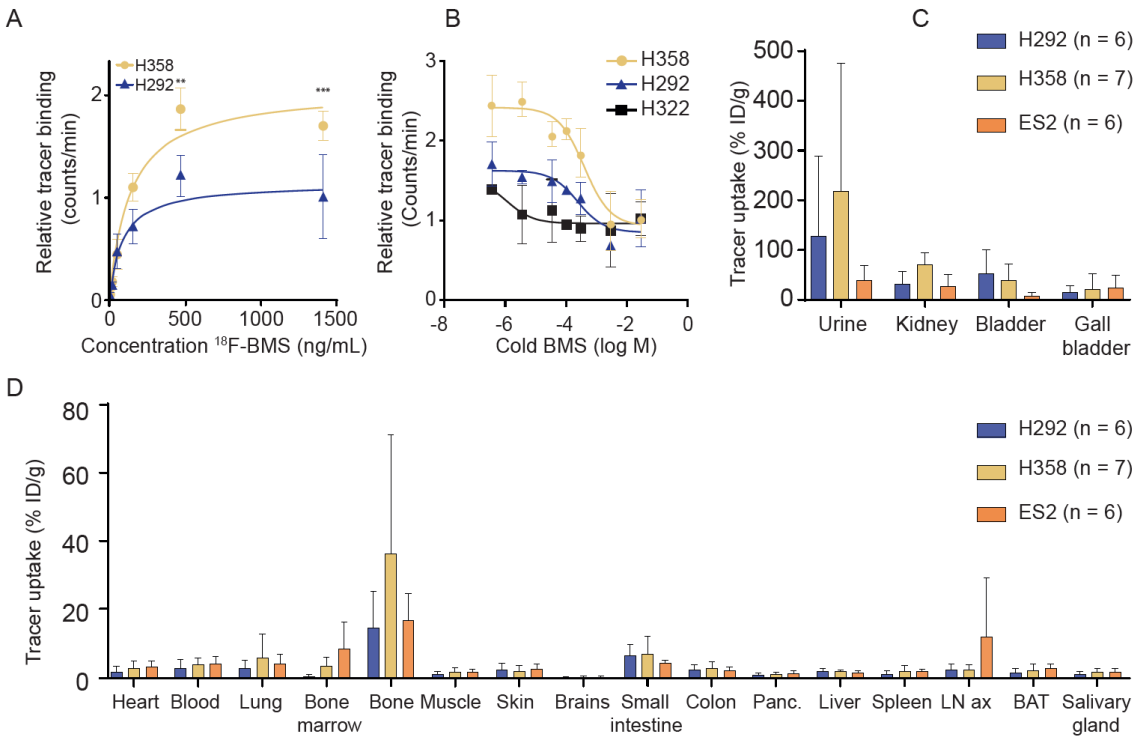
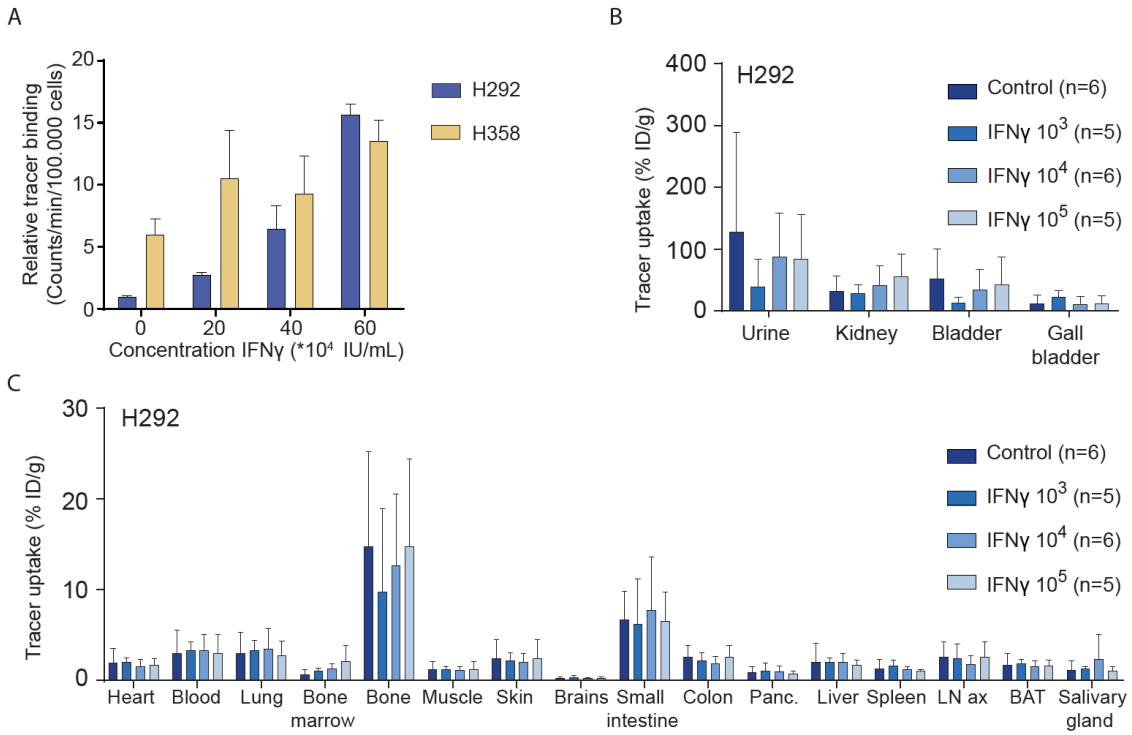
Tracer Production. ^{18}F-BMT-187144 was produced as a precursor for the synthesis of ^{18}F-BMS-986192 using a Zymark robotic system. ^{18}F-fluoride was produced by irradiation of ^{18}O-H_2O with an IBA Cyclone 18 twin cyclotron via the ^{18}O(p,n)^{18}F nuclear reaction. The aqueous ^{18}F-fluoride was passed through a Sep-Pak light QMA anion exchange cartridge (Waters, Netherlands) to recover the ^{18}O-enriched water. ^{18}F-fluoride was then eluted from the cartridge with 1 mg of potassium carbonate (K_2CO_3, Sigma-Aldrich) in 1 mL of water for injections (in-house) and collected in a vial with 15 mg of Kryptofix [2.2.2] (Merck). To the vial, 1 mL of dry acetonitrile (MeCN, Rathburn) was added and the solvents were evaporated at 130 °C. The radioactive residue (^{18}F-KF-Kryptofix complex) was dried three times by addition and evaporation of anhydrous MeCN (3x 0.5 mL at 130 °C). To the dried ^{18}F-KF-Kryptofix complex, 0.5 mL of BMT-180478 (4 mg/ml in DMSO, Bristol-Meyers Squibb) was added and was allowed to react at 120°C for 10 minutes. The mixture was then diluted in 1.5 mL of water for injections and purified by high-performance liquid chromatography (HPLC) using an Elite LaChrom Hitachi L-7100 pump system with a Luna column (5 µm, 250 mm × 10 mm) equipped with both ultraviolet (UV) detection (Elite LaChrom VWR L-2400 UV detector set at 254 nm; Hitachi) and a Bicron radioactivity monitor. The product was eluted using a mobile phase of 32% MeCN in water with 0.1% trifluoroacetic acid (TFA, Sigma-Aldrich) and a flow rate of 4.6 mL/minute. The radioactive product, with a retention time of ~22 minutes, was collected in 80 mL water. The solution was then applied to a SepPak tC18 cartridge (Waters) and washed twice with 5 mL of water. The final product was eluted with 2 mL of ethanol and collected in a 2.5 mL conical vial. Then ^{18}F-BMT-187144 was transferred to another hot cell equipped with a PharmTracer Eckert & Ziegler synthesis module. After drying of ^{18}F-BMT-187144, 0.3 mL of a solution of BMT-

192920 precursor (4 mg/mL in DMSO, Bristol-Meyers Squibb) was added, followed by the addition of 0.1 mL water for injections. The mixture was allowed to react at 40 °C for 40 minutes. After cooling to 25 °C, the reaction mixture was transferred to the HPLC injection vial. The reaction vial was then washed with 1 mL of water for injections, which was then also transferred to the HPLC injection vial. The diluted reaction mixture was purified by HPLC using a Yarra SEC-3000 column (5 µm, 300 mm × 7.8 mm) and 100% phosphate buffered saline as mobile phase with a flow rate of 1.2 mL/minute. ^{18}F-BMS-986192, with a retention time of approximately 10 minutes, was collected into a 25 mL sterile vial (Mallinckrodt) via a sterilization filter (Millex-LG filter, 25 mm diameter, 0.2 µm pore size, polytetrafluoroethylene membrane, Millipore). An additional 6 mL phosphate buffered saline was added to the sterile vial to obtain a total volume of approximately 8 mL. Ultra-performance liquid chromatography was used for analysis of (radio)chemical purity, radiochemical identity and molar activity. For this, a Waters Acquity H-Class system and a BEH Phenyl column (1.7 µm; 3.0 mm x 50 mm) was used, equipped with both an UV detector (operated at 280 nm) and a radioactivity detector (Berthold FlowStar LB513, Mx50-6 flow cell). Gradient elution with a mixture of 0.1% aqueous TFA in ultrapure water (solvent A) and 0.1% TFA in mass spectrometry-grade acetonitrile (solvent B) was performed at a flow of 0.8 mL/min. The following gradient profile was used: 0-6 min 10-50% B, 6-8 min 50-70% B, 8-10 min 70-10% B. Retention times were 3.1 min for ^{18}F-BMT-187144 and 5.3 min for ^{18}F-BMS-986192.

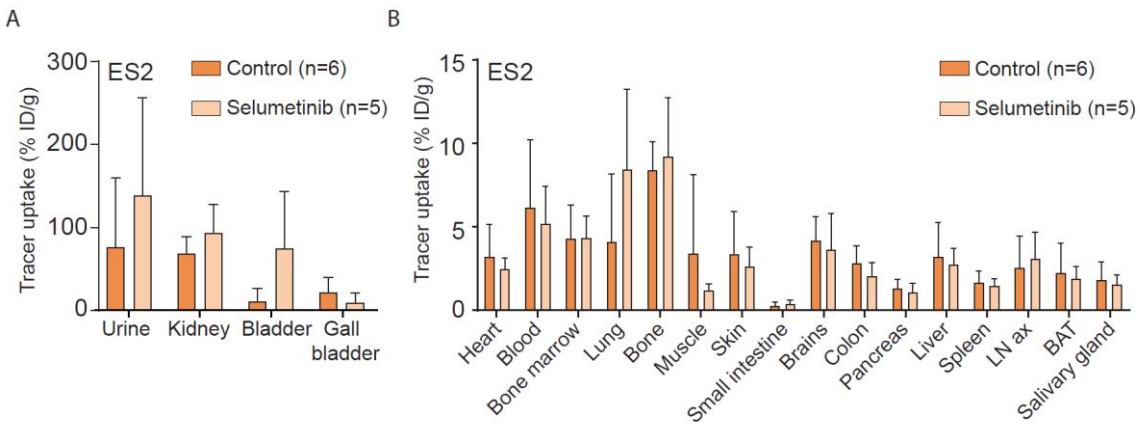


SUPPLEMENTAL FIGURE 1. *In vitro* tracer binding and biodistribution of ^{18}F-BMS-986192 in xenograft models. **(A)** ^{18}F-BMS-986192 was added to H292 or H385 cells and incubated for 60 minutes at 37°C. After washing, the remaining bound counts were measured using a gamma counter. Binding assays were performed in triplicate. Data is expressed relative compared to H292 with the highest tracer concentration. Differences were tested using ANOVA with bonferroni's multiple comparisons test, ** $p < 0.01$, *** $p < 0.001$. **(B)** H358, H292 and H322 cells were incubated with 1 MBq (corresponding to 167 ng) ^{18}F-BMS-986192 for 60 minutes at 37°C together with increasing concentrations of unlabeled precursor. After washing, bound counts were measured using a gamma counter. Binding assays were performed in triplicate and data was expressed relative to the signal of the highest blocking dose. **(C)** A 60 minute dynamic PET scan was performed using ^{18}F-BMS-986192 in BALB/c nude mice with established H292, H358, or ES2 xenograft tumors, followed by *ex vivo* biodistribution studies. Tracer uptake in

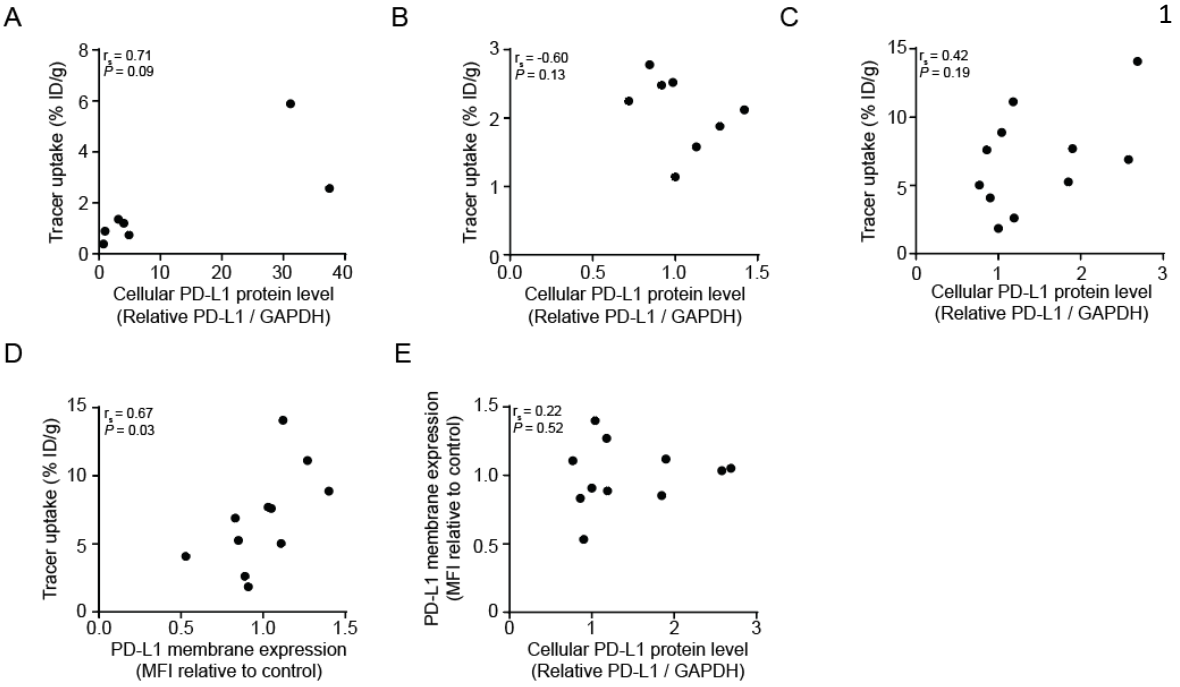
excretion organs and **(D)** other organs was assessed by measuring counts per minute in a gamma counter. Uptake is expressed as percentage of injected dose per gram (%ID/g). Panc. = pancreas, LN ax = axial lymph node, BAT = brown adipose tissue. Data is presented as mean +/± SD.



SUPPLEMENTAL FIGURE 2. IFNγ does not influence biodistribution of ^{18}F-BMS-986192 *in vivo*. **(A)** H292 and H358 cells were treated with a range of IFNγ concentrations for 24 hours. Tracer binding/100,000 cells was measured using a gamma counter and expressed relatively to untreated H292. BALB/c nude mice with established H292 xenograft tumors were randomized between control and different IFNγ doses (n= 5/6 per group). After 3 days treatment by intraperitoneal injection, a 60-min dynamic PET scan was performed using ^{18}F-BMS-986192, followed by *ex vivo* biodistribution studies. Tracer uptake in **(B)** excretion organs and **(C)** other organs was assessed *ex vivo* by measuring counts per minute in a gamma counter. Uptake is expressed as percentage of injected dose per gram (%ID/g). Panc. = pancreas, LN ax = axial lymph node, BAT = brown adipose tissue. Data is presented as mean + SD.



SUPPLEMENTAL FIGURE 3. Selumetinib does not influence biodistribution of ^{18}F-BMS-986192 *in vivo*. BALB/c nude mice with established ES2 xenograft tumors were randomized between control and selumetinib treatment (n= 5/6 per group). After 24 hours treatment by oral gavage, a 60-min dynamic PET scan was performed using ^{18}F-BMS-986192, followed by *ex vivo* biodistribution studies. Tracer uptake in **(A)** excretion organs and **(B)** other organs was assessed *ex vivo* by measuring counts per minute in a gamma counter. Uptake is expressed as percentage of injected dose per gram (%ID/g). LN ax = axial lymph node, BAT = brown adipose tissue. Data is presented as mean + SD.



SUPPLEMENTAL FIGURE 4. Relation of PD-L1 protein and membrane expression with tracer uptake. **(A)** Correlation of PD-L1 protein levels in Figure 1D, with *ex vivo* tracer uptake. **(B)** Correlation of PD-L1 protein levels in Figure 2C, with *ex vivo* tracer uptake. **(C)** Correlation of PD-L1 protein levels in Figure 3E, with *ex vivo* tracer uptake. **(D)** Correlation of PD-L1 membrane expression in Figure 3C, with *ex vivo* tracer uptake. **(E)** Correlation of PD-L1 membrane expression in Figure 3C with PD-L1 protein levels in level 3E. r_s = Spearman's correlation.