Production of ^{18}F-FBnTP

To ^{18}F-fluoride, azeotropically dried in the presence of Kryptofix and K_2CO_3, 4-trimethylammoniumbenzaldehyde (10 mg) in dimethylformamide (DMF, 400 µL) was added, and heated to 110 °C for 10 min. Sodium borohydrate (15 mg) in H_2O (500 µL) was added to the cooled reaction vessel and reacted at room temperature for 5 min. Conc. hydroiodic acid (1 mL) was added to the same reaction vial and reacted for 10 min. The reaction mixture was diluted with H_2O and passed through a C-18 cartridge. The cartridge was washed with sodium thiosulphate/K_2CO_3 solution and water before elution with ACN through molecular sieves and a sodium sulphate drying cartridge, into a vial containing triphenyl phosphine (21 mg) in ACN (Figure 1). The sealed vial was heated to 110 °C for 10 min before being allowed to cool to room temperature. ^{18}F-FBnTP was then purified by HPLC and reformulated in 10 % ethanol, saline solution. Purity was confirmed by analytical radio-HPLC. Specific activity 15.0 ± 12.6 GBq/µmol.

Production of ^{18}F-Mitophos

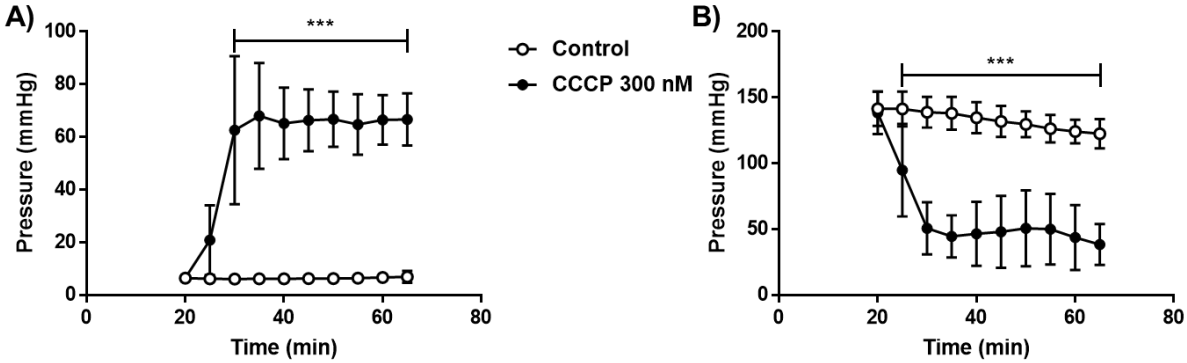
Tosyl ethyl azide in acetonitrile (ACN) was added to ^{18}F-fluoride, azeotropically dried in the presence of Kryptofix and K_2CO_3, and heated for 5 min at 105 °C. ^{18}F-Fluoroethyl azide was distilled into a vial containing $CuSO_4 \cdot 5H_2O$ (56 µL, 0.4 mmol/L), tris(benzyltriazolylmethyl)amine (TBTA) (1 mg), sodium ascorbate (40 mg) 3-but-3-ynyl (tris-3,5-dimethylphenyl)phosphonium bromide (2 mg) in H_2O:EtOH 3:1 solution. The reaction was left for 5 min after distillation had completed and reaction mixture purified by isocratic high performance liquid chromatography (HPLC) (ACN: ammonium formate buffer, pH 4 100 mmol/L, 50:50) (Figure 1). ^{18}F-Mitophos was reformulated in 10 % EtOH and saline and purity confirmed by analytical radio-HPLC. Specific activity 24.6 ± 18.3 GBq/µmol.

Target Organ	Alpha	Beta	Photon	Total	EDE Cont.	ED Cont.
Adrenals	0.00E+00	7.40E-02	1.95E-02	9.34E-02	5.61E-03	2.34E-04
Brain	0.00E+00	2.94E-04	2.17E-03	2.46E-03	0.00E+00	6.15E-06
Breasts	0.00E+00	8.89E-04	3.60E-03	4.49E-03	6.74E-04	2.25E-04
Gallbladder Wall	0.00E+00	8.89E-04	1.81E-02	1.90E-02	0.00E+00	0.00E+00
LLI Wall	0.00E+00	7.88E-03	2.11E-02	2.90E-02	0.00E+00	3.47E-03
Small Intestine	0.00E+00	1.53E-01	3.77E-02	1.91E-01	1.14E-02	4.77E-04
Stomach Wall	0.00E+00	*2.29E-02*	1.64E-02	*3.93E-02*	0.00E+00	*4.71E-03*
ULI Wall	0.00E+00	1.08E-02	4.06E-02	5.14E-02	3.09E-03	1.29E-04
Heart Wall	0.00E+00	2.38E-02	1.12E-02	3.50E-02	0.00E+00	0.00E+00
Kidneys	0.00E+00	9.37E-02	3.85E-02	1.32E-01	7.93E-03	3.30E-03
Liver	0.00E+00	4.87E-03	1.08E-02	1.56E-02	0.00E+00	7.81E-04
Lungs	0.00E+00	8.11E-03	6.33E-03	1.44E-02	1.73E-03	1.73E-03
Muscle	0.00E+00	1.19E-03	7.27E-03	8.46E-03	0.00E+00	2.11E-05
Ovaries	0.00E+00	8.89E-04	2.74E-02	2.83E-02	7.08E-03	5.66E-03
Pancreas	0.00E+00	2.80E-03	1.42E-02	1.70E-02	0.00E+00	4.24E-05
Red Marrow	0.00E+00	5.45E-03	1.12E-02	1.66E-02	2.00E-03	2.00E-03
Osteogenic Cells	0.00E+00	1.79E-02	7.92E-03	2.58E-02	7.75E-04	2.58E-04
Skin	0.00E+00	8.89E-04	3.64E-03	4.52E-03	0.00E+00	4.52E-05
Spleen	0.00E+00	2.75E-02	1.95E-02	4.70E-02	0.00E+00	1.18E-04
Testes	0.00E+00	8.89E-04	4.68E-03	5.57E-03	0.00E+00	0.00E+00
Thymus	0.00E+00	3.32E-03	4.96E-03	8.27E-03	0.00E+00	2.07E-05
Thyroid	0.00E+00	8.78E-02	1.58E-02	1.04E-01	3.11E-03	5.18E-03
Urinary Bladder Wall	0.00E+00	3.72E-02	2.40E-02	6.13E-02	3.68E-03	3.06E-03
Uterus	0.00E+00	8.89E-04	2.59E-02	2.68E-02	0.00E+00	6.70E-05
Total Body	0.00E+00	4.34E-03	7.99E-03	1.23E-02	0.00E+00	0.00E+00
Effective Dose Equivalent (mSv/MBq)					**4.71E-02**	
Effective Dose (mSv/MBq)						**3.15E-02**

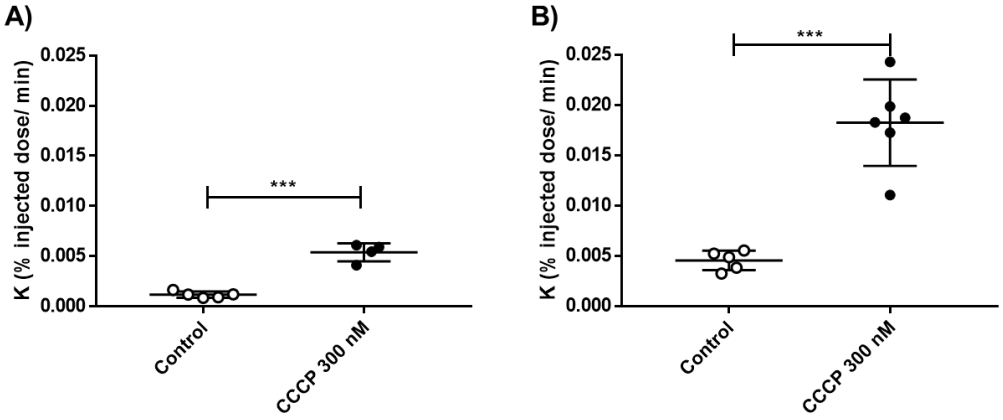
SUPPLEMENTAL TABLE. 1. Dosimetry data for ^{18}F-Mitphos calculated from Sprague Dawley rats (n=3 per timepoint at 5, 15, 30 and 60 min).

Clinical sign	10 mg/kg	15 mg/ kg	20 mg/kg
Piloerection	3/6	5/6	6/6
Hunched posture	1/6	1/6	2/6
Vocalisation	0/6	1/6	0/6
Average BW loss/ %	5.3 ± 2.8	7.0 ± 1.5	6.5 ± 2.4

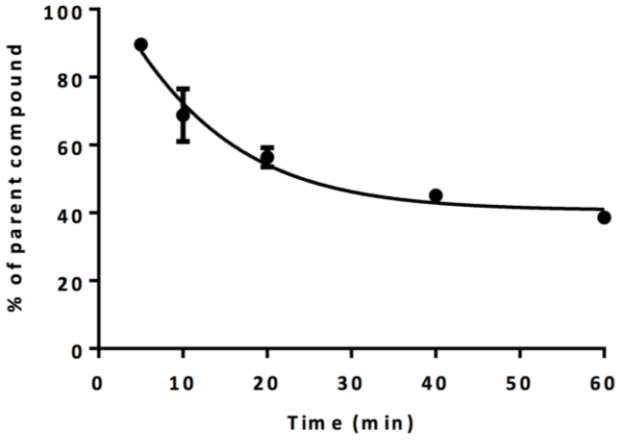
SUPPLEMENTAL TABLE. 2. Adverse effects signs observed for DOX infused animals over the 48 h period post dose. Animals were assessed twice daily and weighed daily. Weight loss is quoted as the total weight loss over the 48 h period.



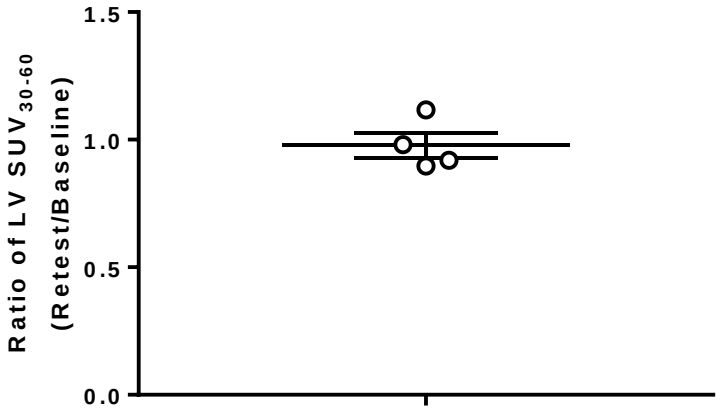
SUPPLEMENTAL FIGURE 1. Cardiac parameters for hearts perfused on the Langendorff perfusion rig. Significant response to both A) LVEDP and B) LVDP are observed upon treatment with CCCP 300 nM from 20 min until the end of protocol showing decreased function of the perfused heart upon CCCP infusion. Data prior to 20 min represents manual perfusion of the heart and stabilisation period and is therefore excluded. Statistical analysis was 2-way ANOVA and Dunnett's post hoc analysis. *** $P<0.001$.



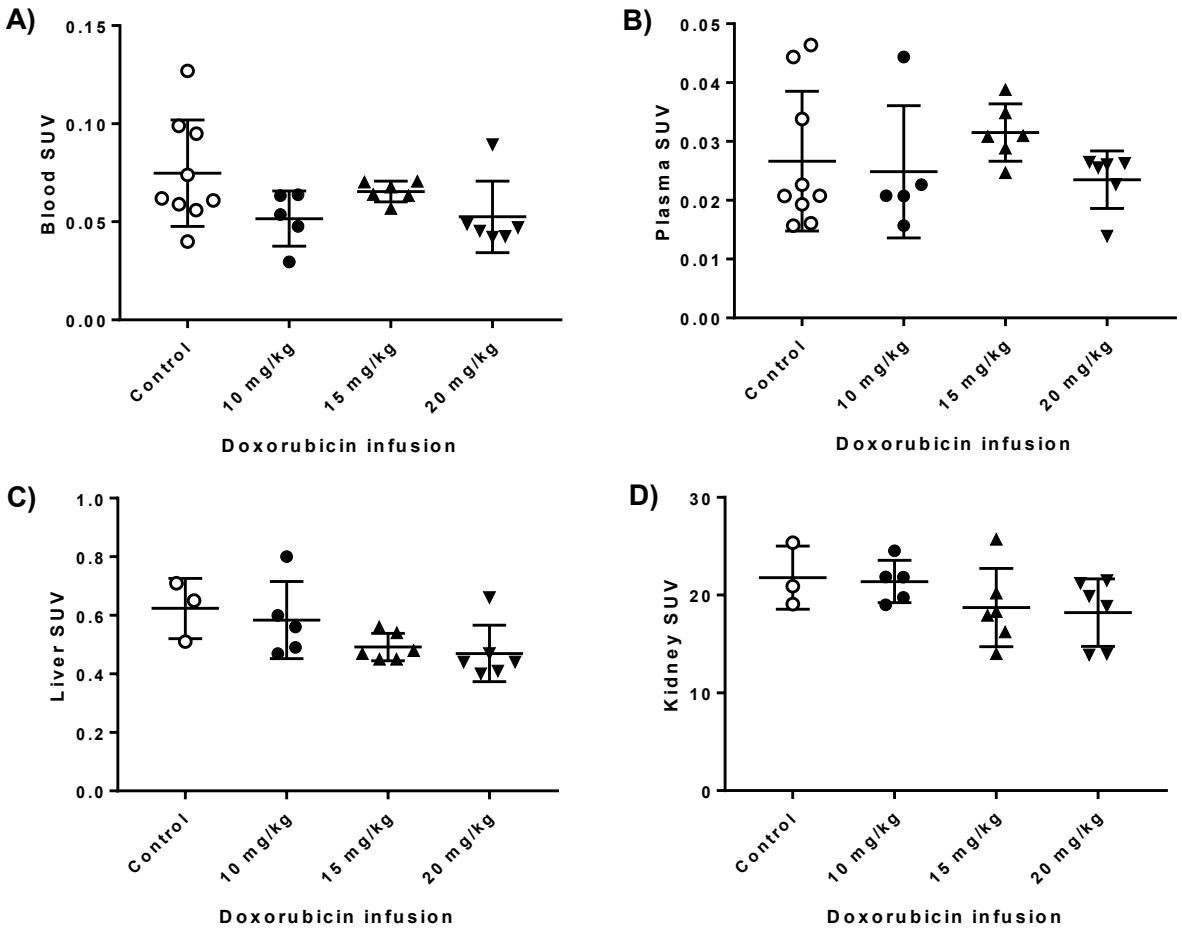
SUPPLEMENTAL FIGURE 2. Washout rate, K, in the Langendorff perfused heart model. K is the least square fit of the exponential washout rate between 2 min post injection and 20 min post injection for A) ^{18}F-Mitophos and B) ^{99m}Tc-Sestamibi. Statistical analysis was 1-tailed student t-test, *** P<0.001.



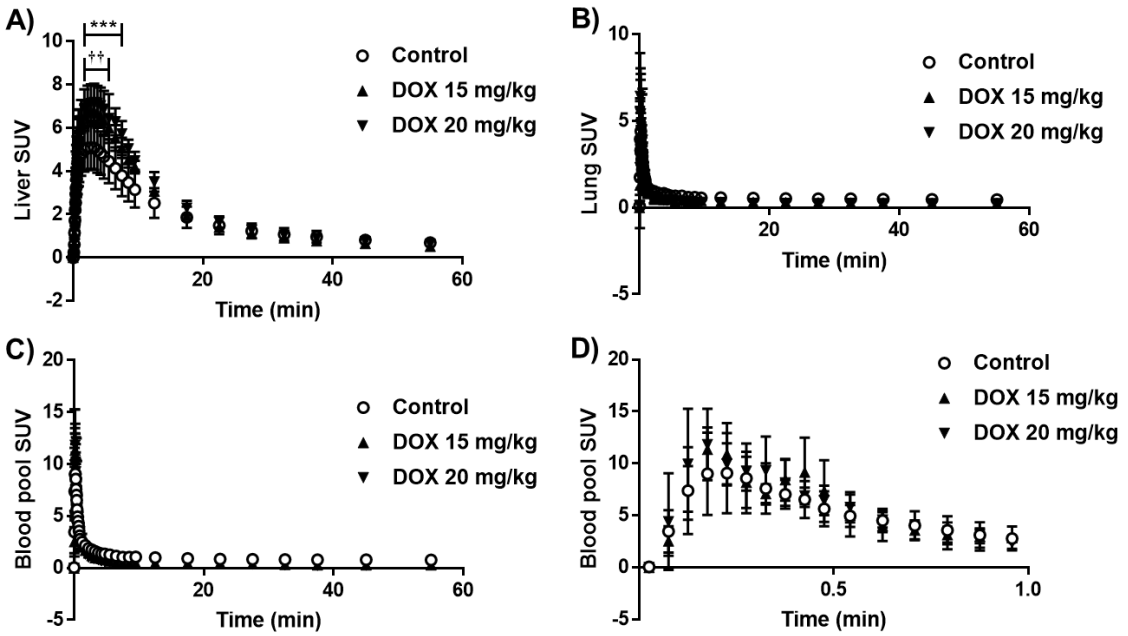
SUPPLEMENTAL FIGURE 3. Metabolism profile of ^{18}F-Mitophos in Sprague Dawley rats (n=2). Plasma was extracted from blood samples and compared to metabolite concentrations via HPLC chromatogram. >90 % activity was recovered from HPLC.



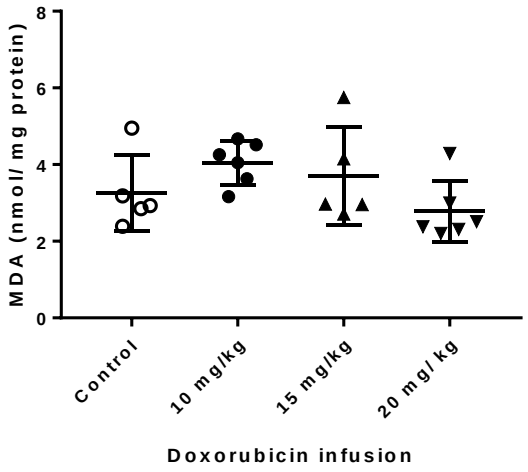
SUPPLEMENTAL FIGURE 4. Test-retest data of ^{18}F-Mitophos uptake in cardiac tissue at 60 min in Sprague Dawley rats. Ratio of left ventricle SUV_{30-60} derived from dynamic scan TAC's (retest/baseline) from two scans 48-72 h apart was used.



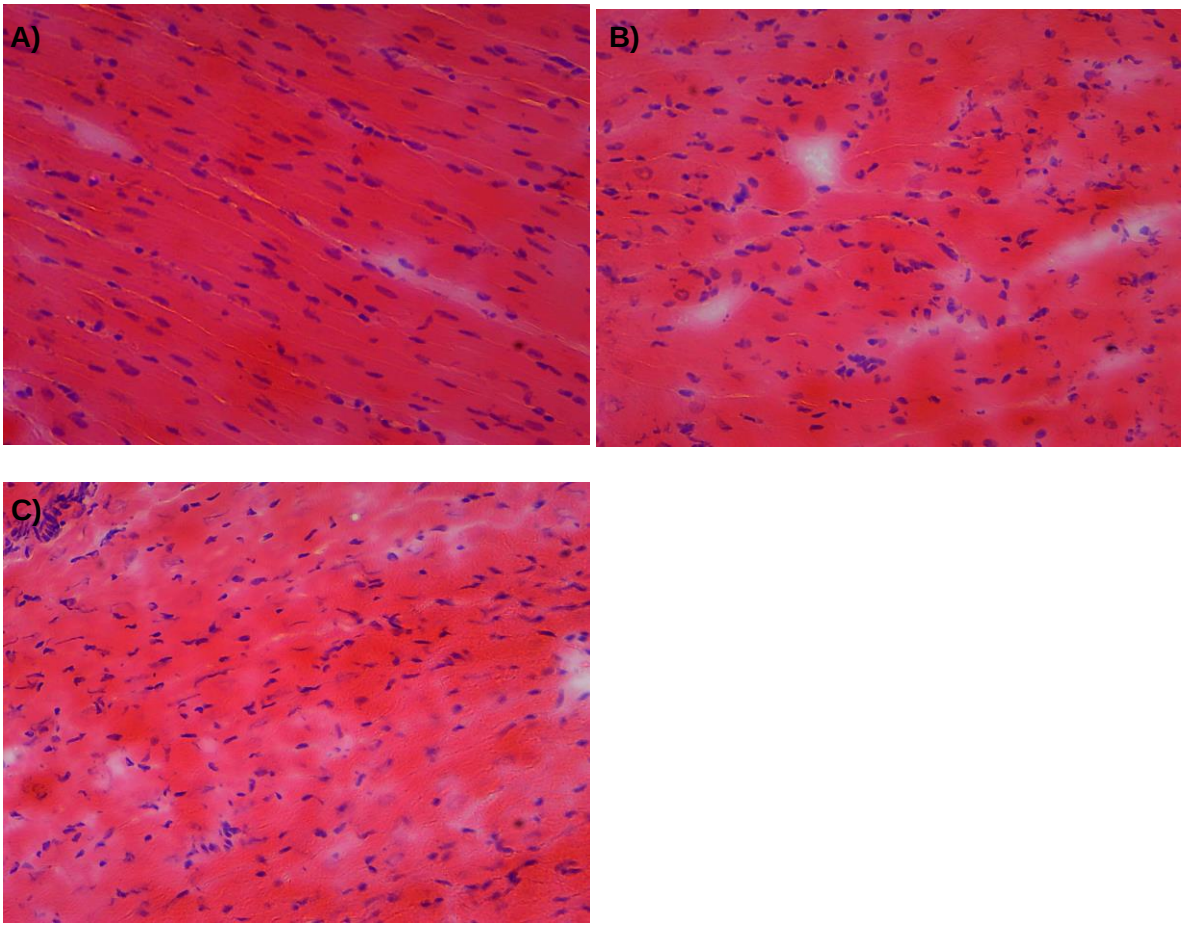
SUPPLEMENTAL FIGURE 5. Cut and count activity at 60 min p.i. of ^{18}F-Mitophos in blood (A), plasma (B), Liver (C) and kidney (D) from control and DOX treated rats. No significant alteration was observed in any tissue group.



SUPPLEMENTAL FIGURE 6. Time activity curves (average SUV ± SD) of ^{18}F-Mitophos in Sprague Dawley rats from A) liver, B) lung, C) blood pool and D) blood pool from the first minute. *** P<0.001 for control compared to DOX 20 mg/kg, †† P<0.01 for control compared to DOX 15 mg/kg *, ** and † confidence intervals were omitted for clarity in A) and occurred only directly adjacent to indicated regions. Blood pool TAC after approximately 1 min must be treated with extreme caution as, due to the proximity and SUV differences to the myocardium, large partial volume effect is observed.



SUPPLEMENTAL FIGURE 7. MDA levels measured in the apex of the cardiac tissue from control and DOX treated groups.



SUPPLEMENTAL FIGURE 8. Example H&E stained longitudinal left ventricle sections (10 µm). A) control tissue, B) moderate damage, C) severe damage. Sections were assessed for myofibrillar loss, cytoplasmic vacuolisation, myocardial disorganisation, inflammatory cell infiltration and haemorrhages.