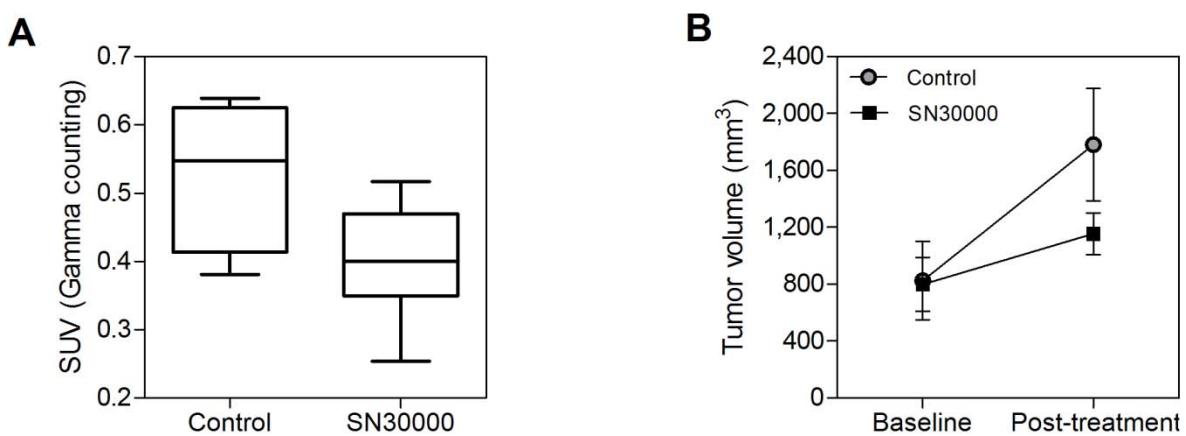
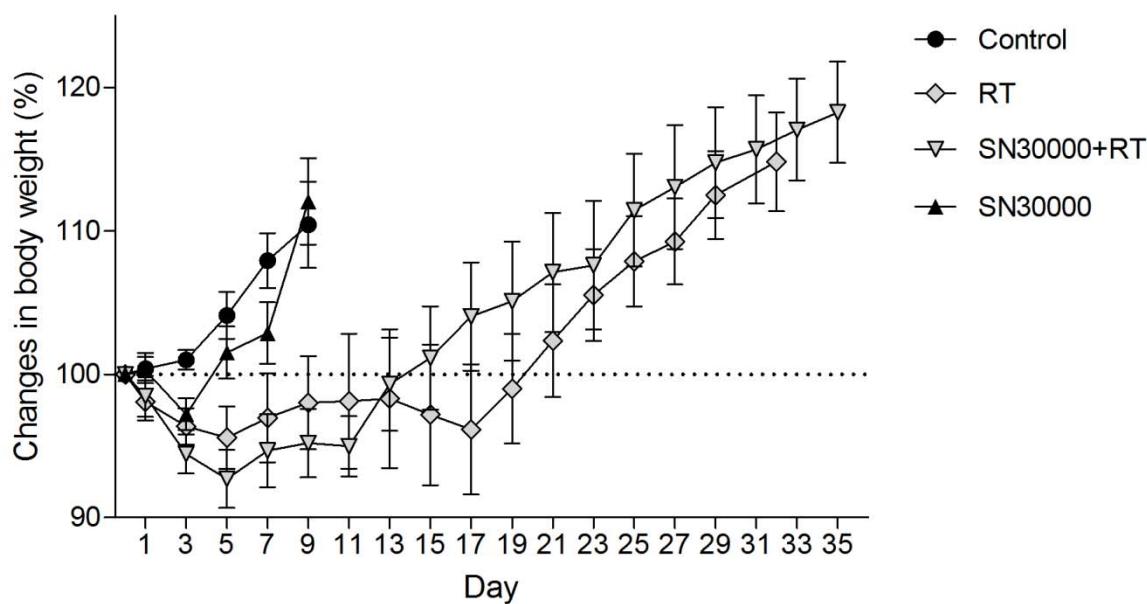


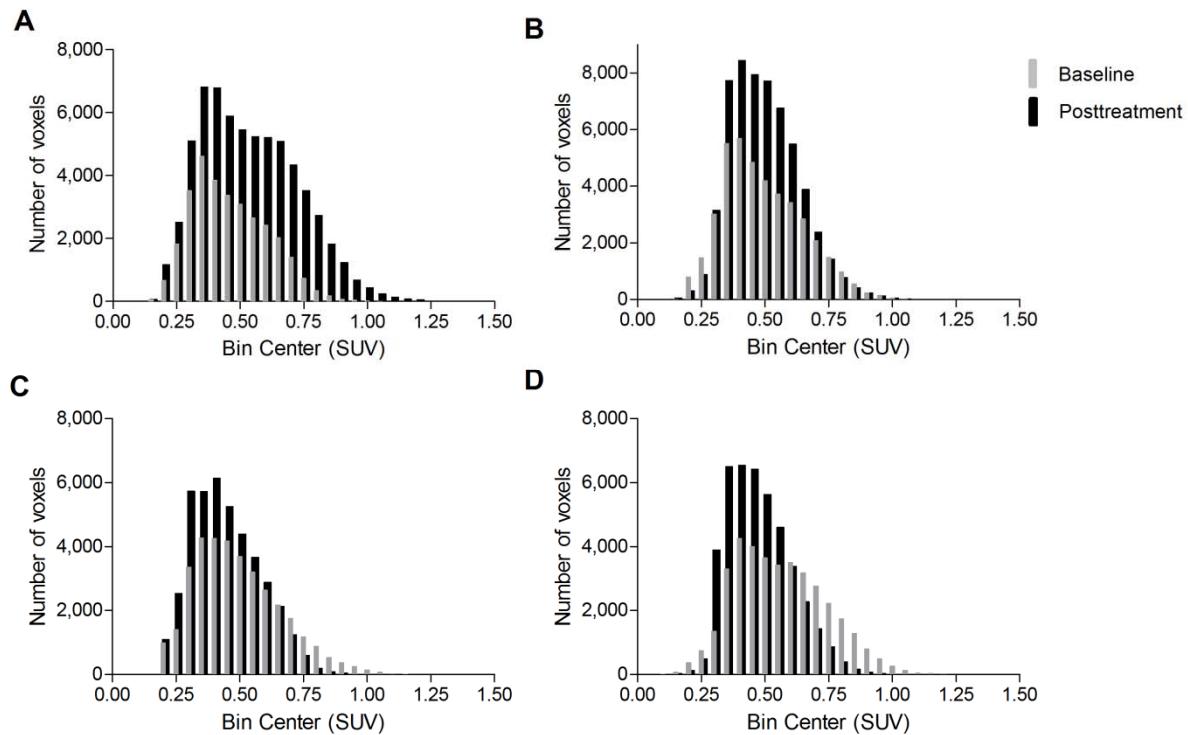
Supplemental Figure 1. Chemical structures of ¹⁸F-EF5 (A) and the hypoxia-activated prodrug SN30000 (B).



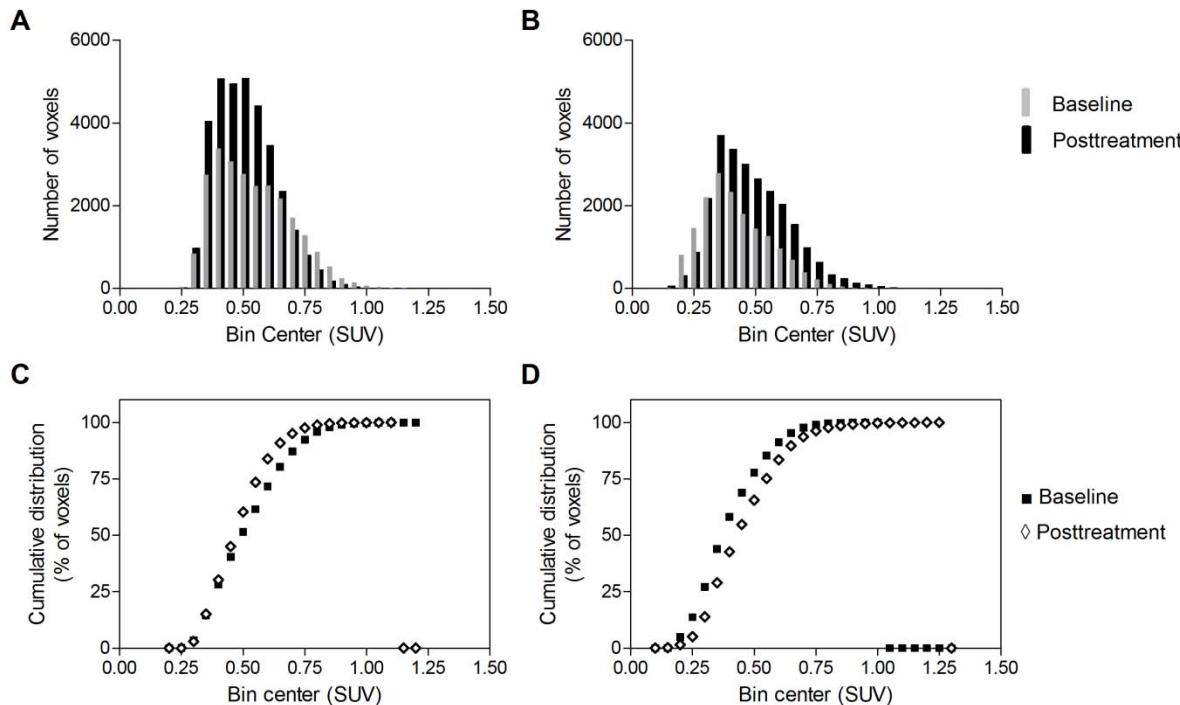
Supplemental Figure 2. (A) Whisker plot showing mean SUVs derived from ex vivo gamma counting of tumors after the post-treatment PET scan in initial feasibility studies. In line with the PET data, the uptake of ^{18}F -EF5 was lower in SN30000 treated tumors compared to saline controls ($P = 0.07$). (B) Changes in tumor volume between pre- and posttreatment ^{18}F -EF5 PET scans.



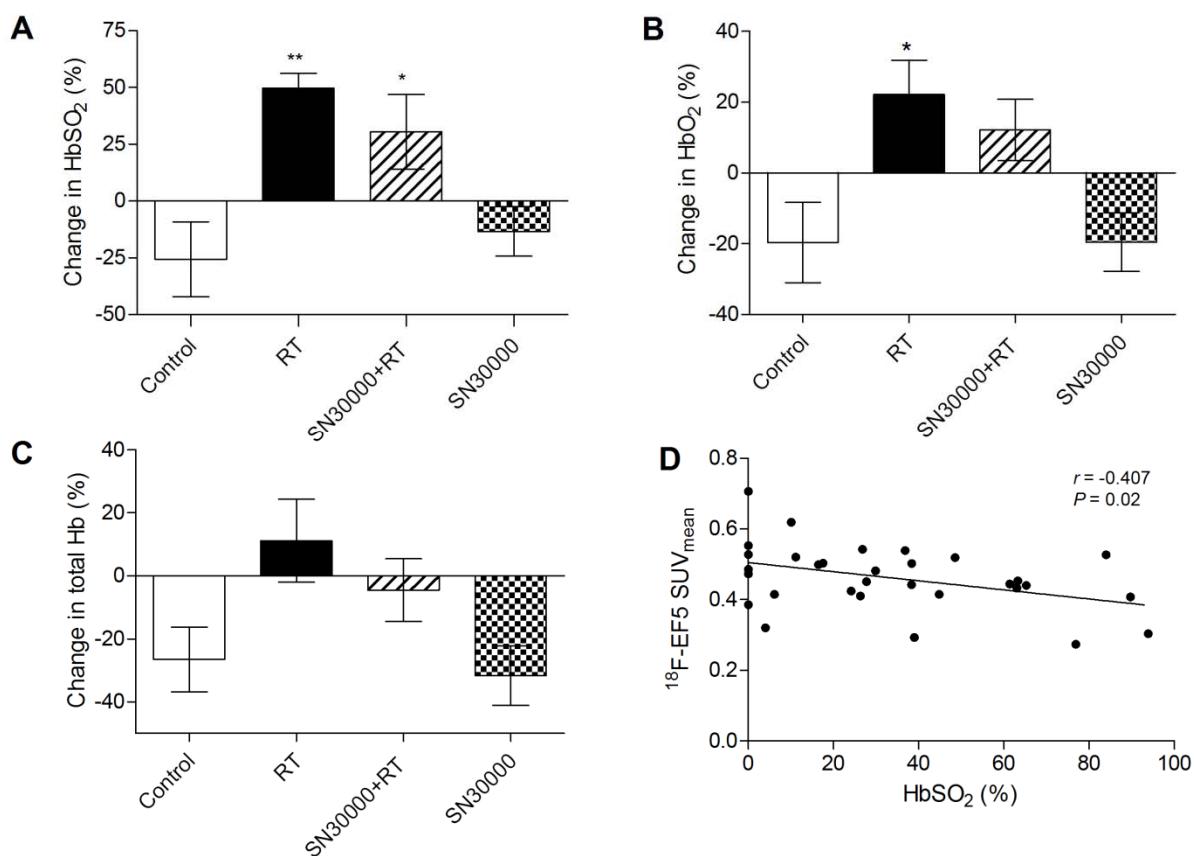
Supplemental Figure 3. Comparison of changes in body weight relative to baseline for animals treated with sham irradiation (control), RT (15 Gy), SN30000 alone (90 mg/kg) or SN30000+RT. Data is presented as mean and SEM for all treatment groups.



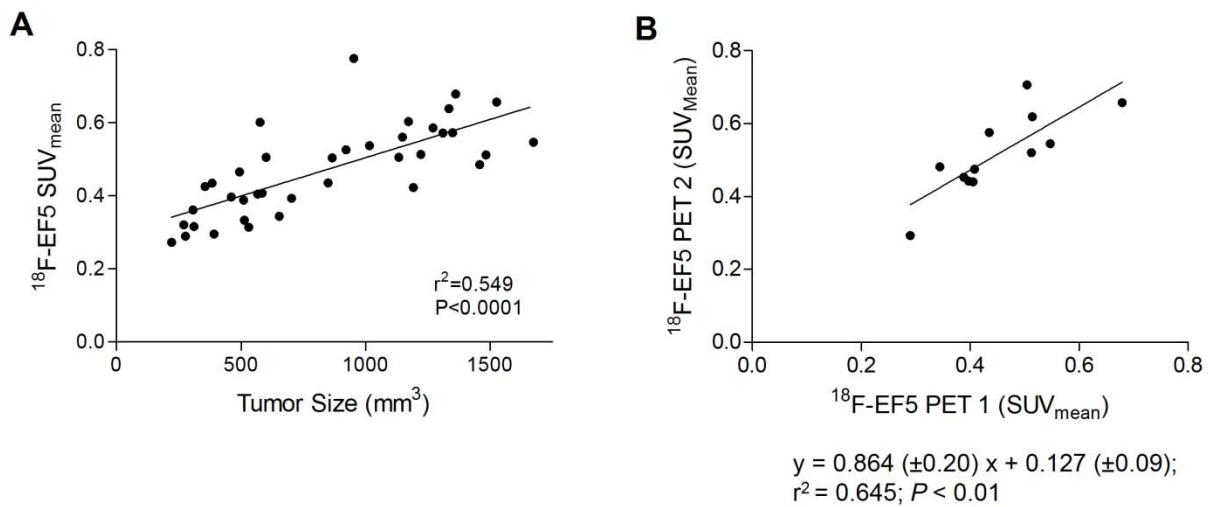
Supplemental Figure 4. Voxel-wise analysis of ^{18}F -EF5 PET imaging data. Histogram plots of voxel intensity arranged in SUV bins of 0.05 and plotted against number of voxels on a pre- and posttreatment basis for Control (A), RT (B), SN30000+RT (C) and SN30000 alone (D) groups.



Supplemental Figure 5. Histogram plots and cumulative distribution curves of ^{18}F -EF5 voxels for tumors treated with SN30000. Histogram plots for tumors that showed reduction in ^{18}F -EF5 uptake (A) vs. tumors that did not show any reduction (B) in the PET signal in the posttreatment scan. Cumulative distribution of voxel intensity for tumors that showed reduction in ^{18}F -EF5 uptake (C) vs. tumors that did not show reduction (D) in ^{18}F -EF5 uptake posttreatment.



Supplemental Figure 6. Comparison of changes (%) in tumor hemoglobin saturation (HbSO₂) (A), oxygen concentration (HbO₂) (B), and total hemoglobin content (C) on day 8 after treatment for Control, RT and SN3000+RT groups, and day 9 for SN3000 alone group. * $P < 0.05$, ** $P < 0.001$; compared to sham-controls. Error bars represent SEM. (D) Correlation of tumor oxygenation status measured by optical spectroscopy with ¹⁸F-EF5 SUV_{mean} for individual tumors in posttreatment scans (Pearson correlation coefficient: -0.407, $P = 0.02$).



Supplemental Figure 7. (A) Scatterplot of ^{18}F -EF5 uptake vs. tumor size for all tumors showing positive correlation between the two ($r^2 = 0.55$). (B) Reproducibility of ^{18}F -EF5 uptake between pretreatment and posttreatment scans in tumors from the control group.

Supplemental Table 1. Average voxel intensities in H460 tumors before and after treatment with SN30000, RT, SN30000+RT or saline (controls) in tumor growth delay studies.

Treatment	SUV _{mean}		SUV _{median}		SUV _{max}	
	Baseline	Post-treatment	Baseline	Post-treatment	Baseline	Post-treatment
Control	0.42 ± 0.08	0.49 ± 0.13*	0.40 ± 0.09	0.48 ± 0.14*	0.95 ± 0.12	1.09 ± 0.26
SN30000	0.47 ± 0.10	0.49 ± 0.05	0.44 ± 0.10	0.47 ± 0.06	0.98 ± 0.14	1.04 ± 0.10
RT	0.44 ± 0.12	0.40 ± 0.10	0.43 ± 0.13	0.39 ± 0.10	0.95 ± 0.26	0.80 ± 0.19
SN30000+RT	0.54 ± 0.10	0.46 ± 0.05*	0.52 ± 0.10	0.44 ± 0.05*	1.21 ± 0.14	0.96 ± 0.16**