Supplementary Information

Quantification of Tumor Vascular Permeability and Blood Volume by Positron Emission Tomography

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Running title: NEB PET tumor vascular permeability

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Table S1. Imaging and therapy regimen

Group (n)	Day 0	8 h	Day 1	Day 2	Day 3	Day 4	Day 7
Group A (16)							
INS-1 (5)	!						
UM-SCC-22B (5)	!		`				
U-87 MG (6)	!						
Group B (11)							
U-87 MG	% !						
Group C (36)							
U-87 MG Treated (18)	$	! #	!#	&	$	! #	! #
U-87 MG Control (18)		! #	! #	&		! #	! #
Group D (24)							
UM-SCC-22B Treated (12)	$		! #		$	! #	! #
UM-SCC-22B Control (12)			! #			! #	! #
Group E (8)							
UM-SCC-22B Treated (4)	@		!		@	!&	
UM-SCC-22B Treated (4)			!			!&	

For bevacizumab therapy response monitoring, tumor bearing mice in treated and control groups were sacrificed for EB extraction right after [^{18}F]FAl-NEB PET at 8 h (n = 8, U-87 MG only), day 1 (n = 8), day 4 (n = 8) and day 7 (n = 8) post-treatment, respectively. **!** [^{18}F]FAl-NEB PET; % Dynamic MRI enhanced with Gd-DTPA; & [^{18}F]FDG PET; $ Bevacizumab treatment; @ Doxorubicin treatment; # Evans blue extraction.

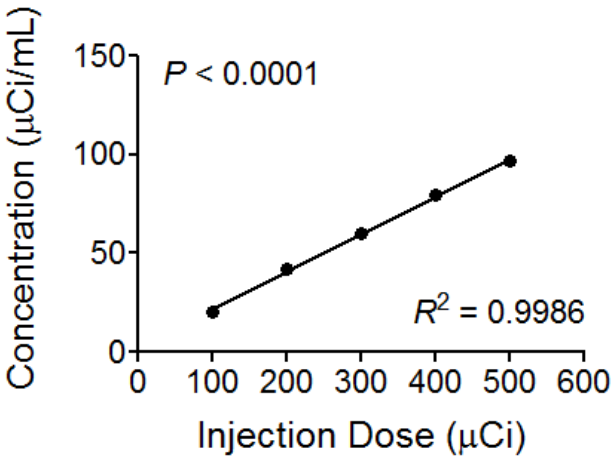

Figure S1. Correlation between the injected dose and tracer concentration in the blood. Differing from other small molecular PET imaging probes, NEB will complex with serum albumin after intravenous injection. Thus, NEB PET can be used to reflect the behavior of serum albumin. In order to eliminate the effect of different injection dose to data analysis, we used %ID/g to perform all the calculations. %ID/g is defined as the tracer concentration divided by the injected dose. For substantiation, we did correlation between the injected dose of [^{18}F]FAl-NEB and *in vivo* tracer concentration. The solid line denotes the best fit linear correlation line. Pearson correlation coefficient R^2, and P value of linear regression are shown.

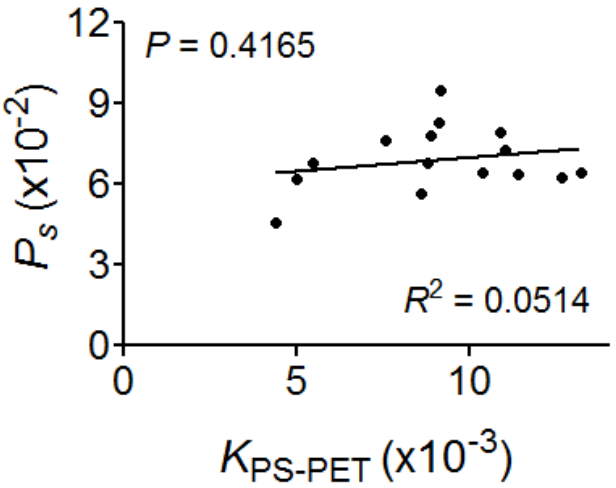

Figure S2. Correlation of P_s with PET-derived kinetic parameter $K_{PS\text{-}PET}$. The compartment modeling of dynamic PET in general computed kinetics analyzes the entire dynamic frames from time zero. The model accounts for tracer's kinetics from its administration to the system, its perfusion, and also its homing to the target area. In this case, the $K_{PS\text{-}PET}$ calculated from the entire dynamic frames (time 0 to 60 min) related not only to the permeability of the [^{18}F]FAl-NEB from vasculature to tumor interstitial space, but also to the perfusion in the blood. Therefore, the P_s calculated from entire dynamic frames would not be able to precisely address the permeability of the studied tracer. Solid line denotes the best fit linear correlation line. Pearson correlation coefficient R^2, and P value of linear regression are shown.

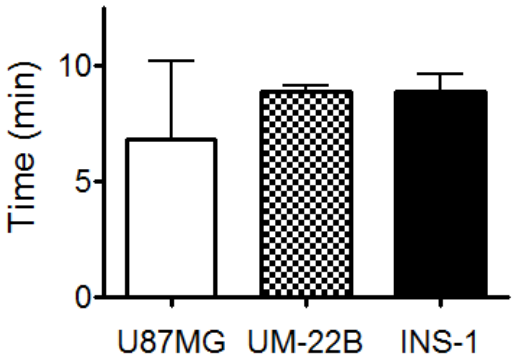

Figure S3. Calculation of equilibrium time. The time when tracer kinetics reach equilibrium (t = T_s) is determined by filtering the tumor TAC data y(t) by Eq. 1 , until the *det* inferiors to certain tolerance ε (e.g. 10% of $\overrightarrow{P_i}$) . $et(\overrightarrow{P_i}, \overrightarrow{P_{i+1}}, \overrightarrow{P_{i+2}}) < \varepsilon$, $(i = 2..N-3)$, where $(\overrightarrow{P_i}) = (y_{i+1} - y_{i-1})/(t_{i+1} - t_{i-1})$. Equilibrium time determined by tumor TAC filtering. All three types of tumor showed similar equilibrium time of 8.21 ± 2.08 min.

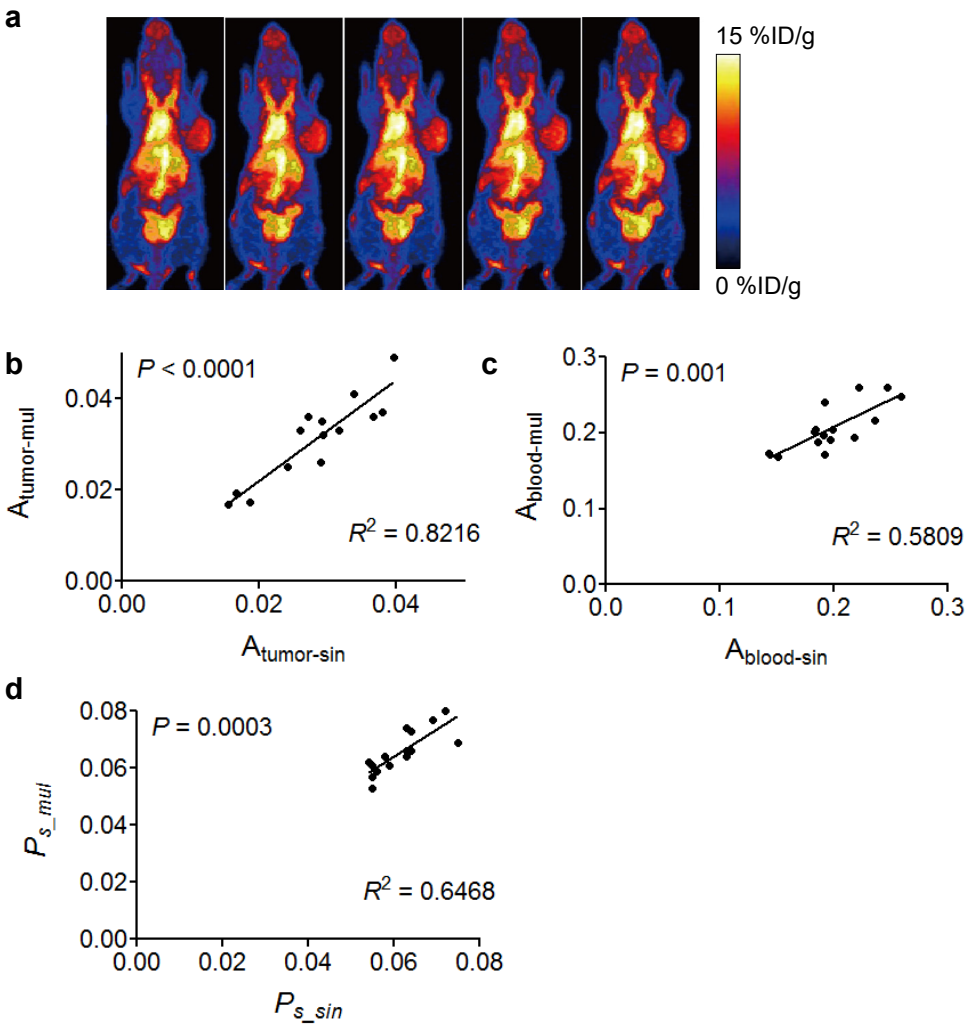

Figure S4. Reconstruction of 10 min static PET. (a) To further simplify the data analysis, we performed reconstruction of the 10 min static data to 5 frames with 2 min per frame. The 10-min static PET image was chosen for reconstruction (U-87 MG, at 60 min p.i.). The 10-min imaging data were split up into 5 frames (10-min dynamic scan from 60-70 min p.i.) **(b-c** Correlation between slope values from tumor **(b)** or blood **(c)** TAC measured by multi-point NEB PET ($A_{tumor\text{-}mul}$ and $A_{blood\text{-}mul}$) with those measured by single-point PET ($A_{tumor\text{-}sin}$ and $A_{blood\text{-}sin}$). **(d)** Correlation of K_p values measured by multi-point NEB PET (P_{s_mul}) with K_p measured by single-point PET (P_{s_sin}). Solid line denotes the best fit linear correlation line. Pearson correlation coefficient R^2, and P value of linear regression are shown.

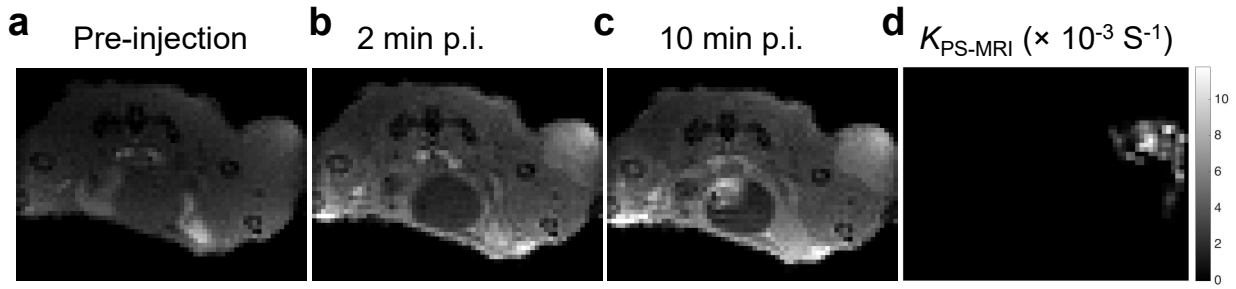

Figure S5. Small animal dynamic MRI and K_{ps} parametric maps. **(a-c)** Representative dynamic contrast-enhanced magnetic resonance images (DCE-MRI) from a U-87 MG tumor bearing mouse. The sequential images show the tumor at different time points after bolus administration of Gd-DTPA. **(d)** The kinetic parametric maps were determined by non-linear least-squares fittings of the time-courses data in each voxel with the generalized kinetic model.

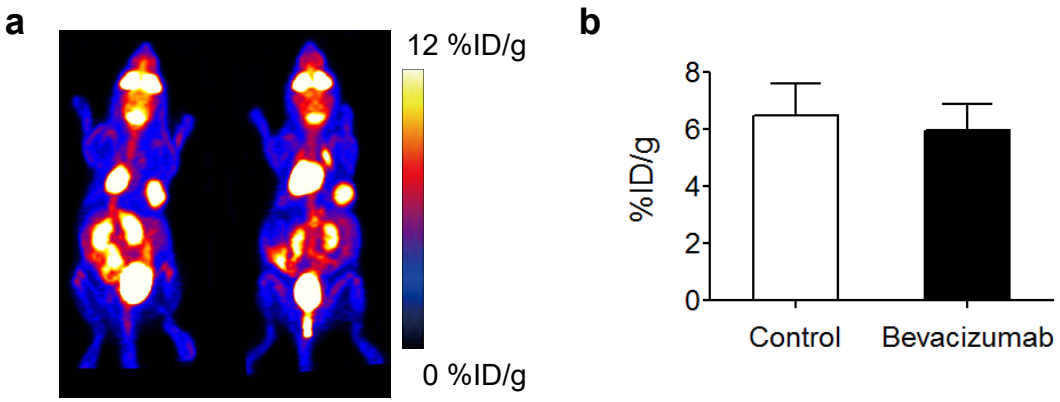

Figure S6. **Bevacizumab therapy response monitoring by [^{18}F]FDG.** [^{18}F]FDG PET scans were carried out on U-87 MG tumor bearing mice after the first dose (day 2 post-treatment) of bevacizumab, to evaluate the changes in tumor metabolism. The 10-min static [^{18}F]FDG PET scans were acquired at 60 min p.i. with an injected dose of 5.55 MBq. Tumor [^{18}F]FDG uptake (%ID/g) was quantified based on ROI analysis. **(a)** [^{18}F]FDG PET images (maximum-intensity-projection) acquired from control and bevacizumab-treated U-87 MG tumor xenografts on day 2 post-treatment. **(b)** U-87 MG tumor uptake of [^{18}F]FDG as quantified from small animal [^{18}F]FDG PET scans.

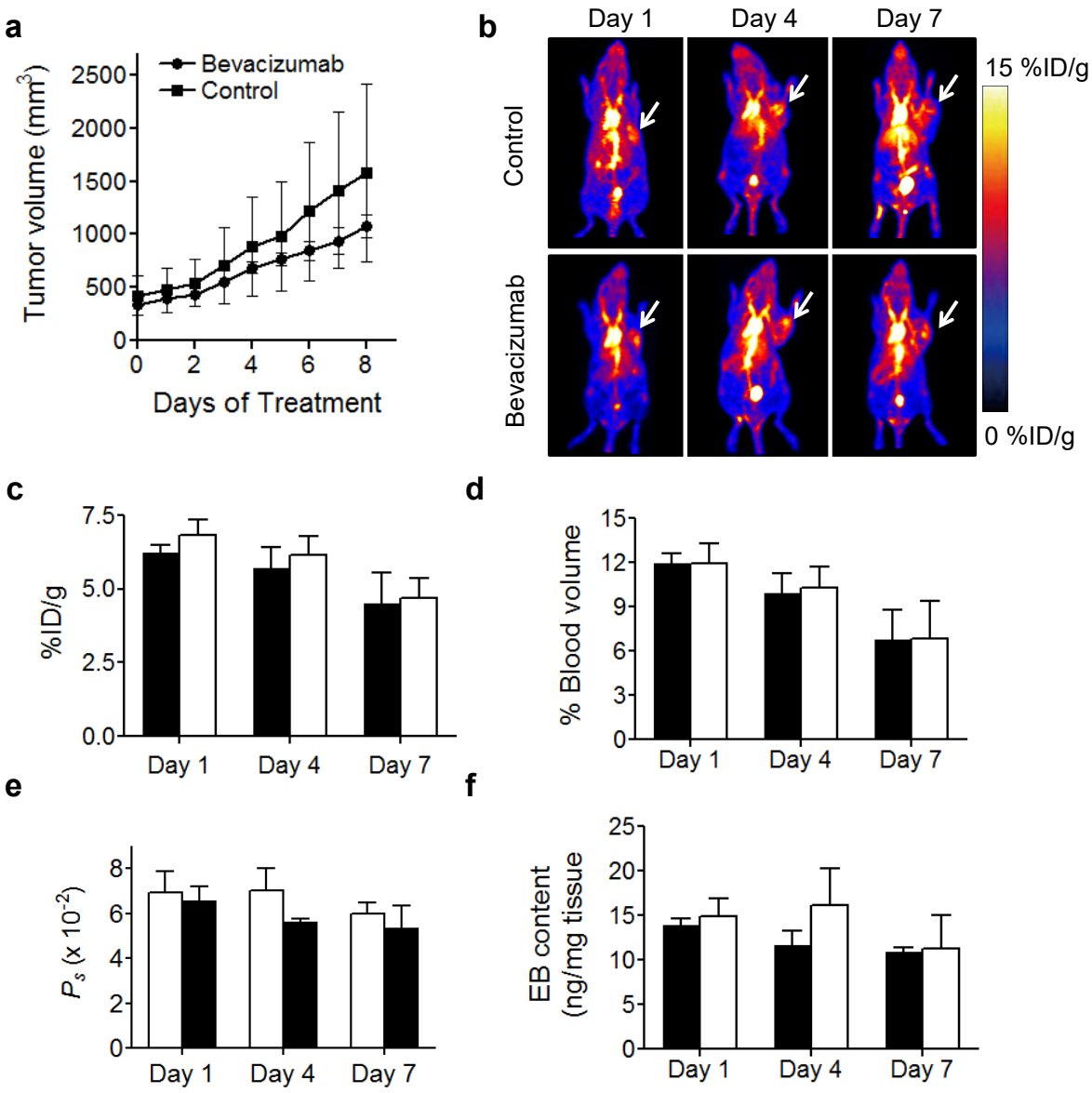

Figure S7. Evaluation of tumor response to bevacizumab therapy. The UM-SCC-22B tumor bearing mice were randomized to receive two doses of bevacizumab (10 mg/kg) or saline intravenously on days 0 and 3. For therapy response monitoring, both control and treated mice received NEB PET on days 1, 4 and 7 post-treatment (n = 4/group). Mice were scanned at 10, 30 and 60 min after intravenous injection of 5.55-7.40 MBq of [^{18}F]FAl-NEB. Tumor [^{18}F]FAl-NEB uptake (%ID/g), blood volume (%), and vascular permeability (P_s) were quantified based on NEB PET images. EB extraction technique was also performed to confirm the changes of tumor vascular permeability observed in NEB PET. **(a)** Tumor growth study of mice with UM-SCC-22B xenografts treated with bevacizumab. **(b)** Representative maximum-intensity-projection NEB PET

images acquired from control and bevacizumab-treated UM-SCC-22B tumor xenografts on days 1, 4 and 7 post-treatment. Images shown here were acquired at 60 min p.i.. **(c-f)** PET Quantification of tumor NEB uptake **(c)**, blood volume **(d)**, P_s values **(e)**, and Evans blue amount **(f)** in bevacizumab-treated and control UM-SCC-22B tumors. Black column = bevacizumab-treated, white column = control.

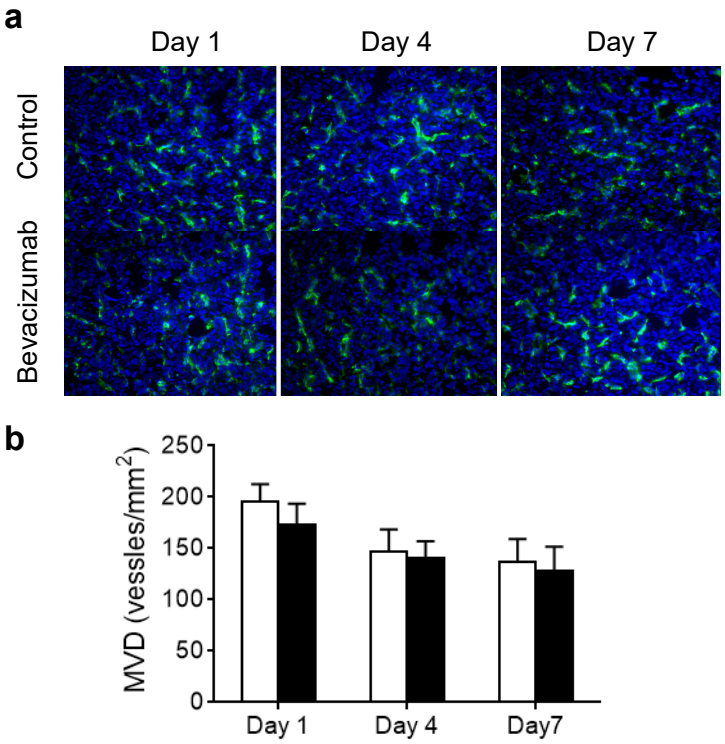

Figure S8. CD31 staining and MVD analysis in UM-SCC-22B tumors treated with bevacizumab. (a) Immunofluorescence staining of CD31 in ex vivo tumor tissue from control and bevacizumab-treated UM-SCC-22B tumor bearing mice on days 1, 4 and 7 post-treatment (magnification×200). **(b)** MVD measurement of control and bevacizumab-treated UM-SCC-22B tumors. Number of vessels counted was divided by field of view to yield MVD, as the number of vessels/mm^2. Black column = bevacizumab-treated, white column = control.