

Multimodal Somatostatin Receptor Theranostics Using [⁶⁴Cu]Cu- /[¹⁷⁷Lu]Lu-DOTA-(Tyr³)octreotate and AN-238 in a Mouse Pheochromocytoma Model

Supplemental data

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Table S3: [⁶⁴Cu]Cu-DOTATATE uptake values in MPC-mCherry tumor-bearing mice as determined in PLI, PET, and ex vivo radiotracer distribution studies; radiotracer uptake was blocked by co-injection of various therapeutic somatostatin analogs at 0.2 μmol/kg; (T) tumor; (% inh.) relative inhibition compared to control; (M) muscle; (H) heart; (B) blood; (L) liver; (K) kidneys; (P) pancreas; data are presented as means ± SEM; significance of differences was tested as compared to control; * $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$.

		control	octreotide	[^{nat} Lu]Lu-DOTATATE	AN-238
<i>Photostimulated luminescence imaging (PLI)</i>					
max UV _{PLI} × 10 ⁵	T (% inh.)	19.3 ± 2.3 (0)	9.1 ± 0.6 (52.9)†	4.9 ± 0.7 (74.7)‡	13.5 ± 1.5 (30.0)*
	M (% inh.)	0.70 ± 0.1 (0)	0.66 ± 0.1 (6.6)	0.64 ± 0.1 (8.5)	0.71 ± 0.1 (-1.4)
	T/M	25.3 ± 2.6	13.8 ± 30.3†	7.6 ± 31.0‡	19.4 ± 31.4*
<i>Positron emission tomography (PET)</i>					
max SUV _{PET}	T (% inh.)	8.2 ± 0.2 (0)	4.1 ± 0.1 (49.3) ‡	1.1 ± 0.1 (86.2)‡	6.6 ± 0.7 (19.3)*
	M (% inh.)	0.1 ± 0.01 (0)	0.1 ± 0.01 (-29.5)	0.1 ± 0.01 (-27.6)	0.1 ± 0.02 (-11.7)
	H (% inh.)	0.3 ± 0.05 (0)	0.2 ± 0.03 (28.3)	0.4 ± 0.1 (-37.6)	0.3 ± 0.1 (-14.9)
	L (% inh.)	1.1 ± 0.2 (0)	0.9 ± 0.1 (11.5)	1.1 ± 0.1 (-5.3)	0.8 ± 0.04 (20.7)
	K (% inh.)	1.4 ± 0.4 (0)	1.7 ± 0.4 (-20.3)	1.2 ± 0.1 (14.3)	1.7 ± 0.2 (-19.1)
	T/M	78.9 ± 4.7	31.5 ± 4.4‡	8.5 ± 0.7‡	58.3 ± 6.0*
<i>Ex vivo radiotracer distribution (RD)</i>					
SUV _{RD}	T (% inh.)	10.9 ± 0.3 (0)	5.2 ± 0.3 (51.8)‡	1.7 ± 0.2 (84.9)‡	7.6 ± 1.1 (30.0)‡
	M (% inh.)	0.1 ± 0.01 (0)	0.2 ± 0.2 (-232)	0.3 ± 0.2 (-250)	0.1 ± 0.03 (-40.9)
	B (% inh.)	0.1 ± 0.01 (0)	0.1 ± 0.01 (10.4)	0.1 ± 0.02 (-0.3)	0.1 ± 0.01 (3.2)
	L (% inh.)	1.7 ± 0.3 (0)	1.5 ± 0.1 (12.5)	1.9 ± 0.2 (-7.7)	1.4 ± 0.1 (20.9)
	K (% inh.)	1.2 ± 0.1 (0)	1.2 ± 0.1 (-4.2)	1.5 ± 0.4 (-28.8)	1.9 ± 0.5 (-57.1)
	P (% inh.)	1.8 ± 0.4 (0)	0.9 ± 0.1 (50.8)*	0.8 ± 0.4 (55.5)*	1.7 ± 0.3 (5.8)
	T/M	79.7 ± 48.9	91.0 ± 13.1	14.4 ± 5.3	89.2 ± 26.3
	T/B	118 ± 8.8	61.5 ± 4.1‡	18.6 ± 2.0‡	84.1 ± 4.9†

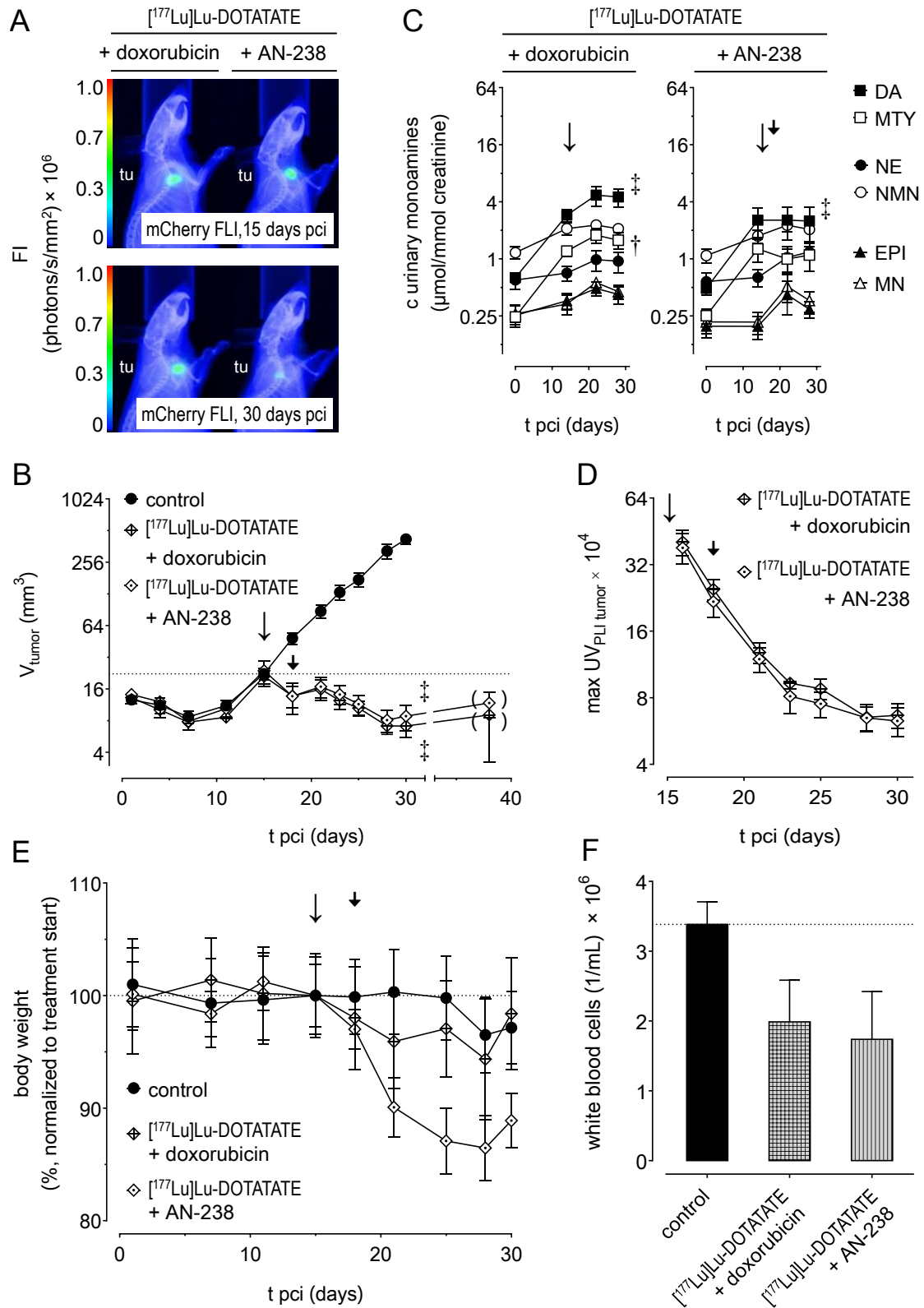


Figure S8: Effects of combined $[^{177}\text{Lu}]\text{Lu-DOTATATE}$ (80 MBq/animal) treatment with doxorubicin (6.9 μmol/kg) or AN-238 (0.2 μmol/kg) in MPC-mCherry tumor-bearing mice; (A) FLI/X-ray overlays after 15 and 30 days pci; (B) monitoring of tumor volume; data points in parentheses: extended follow-up of 3 randomly selected animals; significance of differences as compared to control after 30 days pci; (C) monitoring of renal monoamine excretion; significance of differences as compared to 0 days pci; (C) decay-corrected ^{177}Lu activity in tumors; (E) monitoring of body weight; (F) white blood cell count after 30 days pci; (↓) application of $[^{177}\text{Lu}]\text{Lu-DOTATATE}$ and doxorubicin; (†) application of AN-238; (DA) dopamine; (NE) norepinephrine; (MTY) 3-methoxytyramine; (NMN) normetanephrine; (EPI) epinephrine; (MN) metanephrine; data are presented as means ± SEM; † $p < 0.01$; ‡ $p < 0.001$.

Table S4: Curve fitting parameters describing MPC-mCherry tumor progression and tumor accumulation of ^{177}Lu activity in mice undergoing doxorubicin (6.9 $\mu\text{mol/kg}$), AN-238 (0.2 $\mu\text{mol/kg}$), and [^{177}Lu]Lu-DOTATATE (80 MBq/animal) treatment; (EG) exponential growth (ED) exponential decay; data are presented as means \pm SEM; significance of differences was tested as compared to control: * $p < 0.05$, as compared to doxorubicin: # $p < 0.05$.

	control	doxorubicin	AN-238	[^{177}Lu]Lu-DOTATATE		
				+ doxorubicin	+ AN-238	
<i>Progression of tumor growth (V_{tumor})</i>						
x -range (days pci)	7-30	21-30	21-30	15-30	21-30	21-30
fitting algorithm	EG	EG	EG	ED	ED	ED
R^2	0.996	0.997	0.999	0.935	0.892	0.828
t_D (days)	4.0 ± 0.2	$3.4 \pm 0.1^*$	$4.1 \pm 0.1^\#$	-	-	-
$t_{1/2}$ (days)	-	-	-	2.8 ± 1.1	4.0 ± 1.5	3.9 ± 2.4
<i>Decay-corrected ^{177}Lu activity in tumors ($\max UV_{\text{PLI tumor}}$)</i>						
x -range (days pci)	-	-	-	16-30	21-30	21-30
fitting algorithm	-	-	-	ED	ED	ED
R^2	-	-	-	0.998	0.892	0.828
$t_{1/2}$ (days)	-	-	-	2.3 ± 0.2	2.2 ± 0.3	2.0 ± 0.2

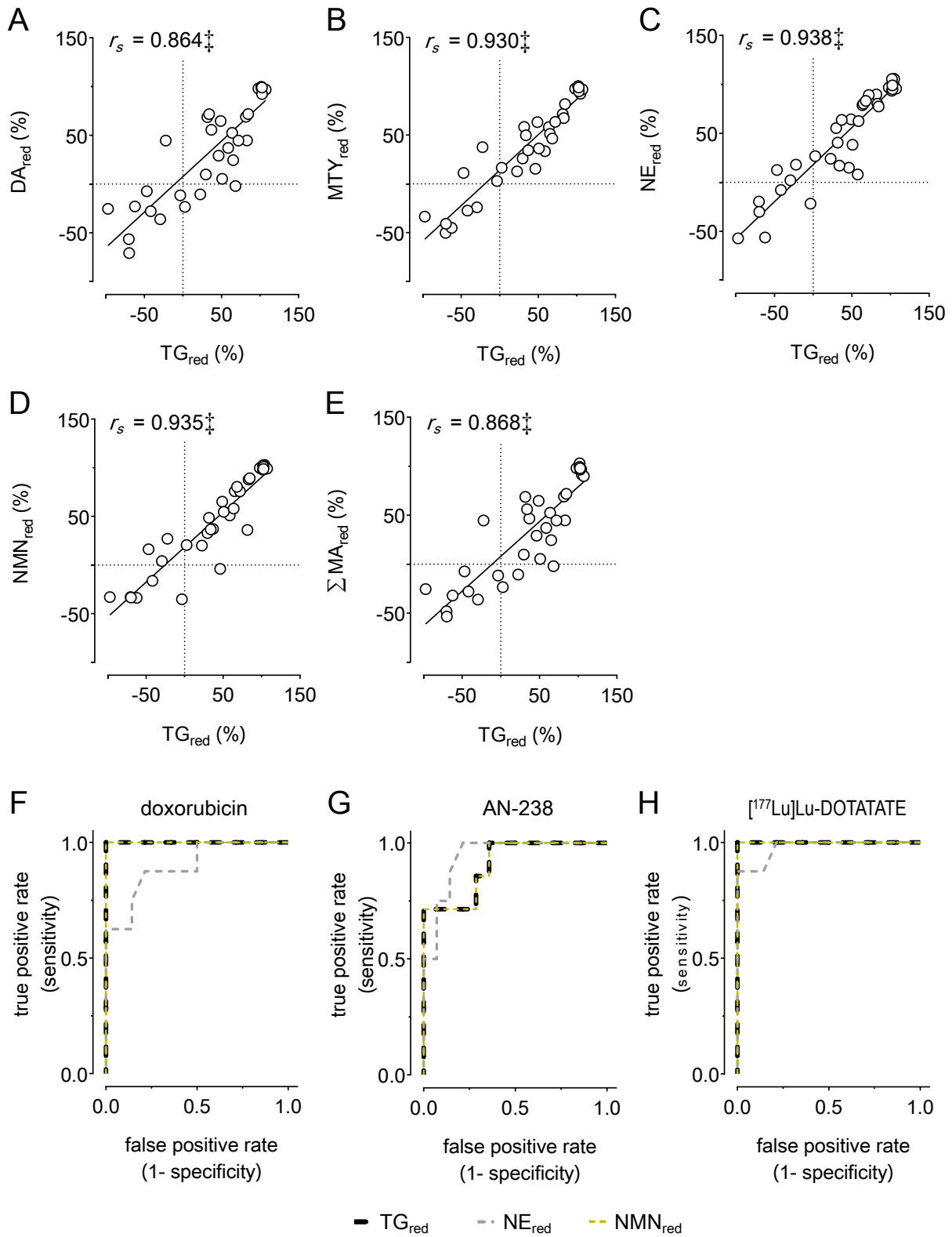


Figure S9: Efficiency of endpoint parameters to evaluate therapeutic outcome in MPC-mCherry-bearing mice after doxorubicin (6.9 $\mu\text{mol/kg}$), AN-238 (0.2 $\mu\text{mol/kg}$), and [¹⁷⁷Lu]Lu-DOTATATE (80 MBq/animal) treatment; (A-E) correlation between the reduction of tumor growth and renal monoamine excretion; (red) reduction compared to control; (TG) tumor growth; (DA) dopamine; (MTY) 3-methoxytyramine; (NE) norepinephrine; (NMN) normetanephrine; (Σ MA) overall monoamines DA + MTY + NE + NMN; (r_s) Spearman's linear correlation coefficient, $\ddagger p < 0.001$; (F-H) ROC curve analysis; areas under curves represent the probability that an animal in a therapy group will have a higher test result of the parameter than a control animal.

Table S5: Probability of endpoint parameters to detect treatment response of MPC-mCherry tumor-bearing mice as determined by ROC curve analysis; (red) reduction compared to control; (TG) tumor growth; (DA) dopamine; (MTY) 3-methoxytyramine; (NE) norepinephrine; (NMN) normetanephrine; (Σ MA) overall monoamines DA + MTY + NE + NMN; data are presented as area under curve (AUC) with [95% confidence interval]; AUC represents the probability that an animal in a therapy group will have a higher test result of the endpoint parameter than a control animal; * $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$; p indicates whether an endpoint parameter significantly discriminates animals in therapy groups from controls.

therapeutic endpoint parameter	doxorubicin	AN-238	[¹⁷⁷ Lu]Lu-DOTATATE
TG _{red}	1 [1-1]‡	0.91 [0.77-1.04]†	1 [1-1]‡
DA _{red}	0.64 [0.37-0.91]	0.76 [0.54-0.97]	1 [1-1]‡
MTY _{red}	0.80 [0.54-1.06]*	0.87 [0.71-1.02]†	1 [1-1]‡
NE _{red}	0.90 [0.76-1.04]†	0.94 [0.85-1.03]‡	0.98 [0.92-1.03]‡
NMN _{red}	1 [1-1]‡	0.91 [0.77-1.04]†	1 [1-1]‡
Σ MA _{red}	0.64 [0.37-0.919]	0.80 [0.60-0.99]*	1 [1-1]‡