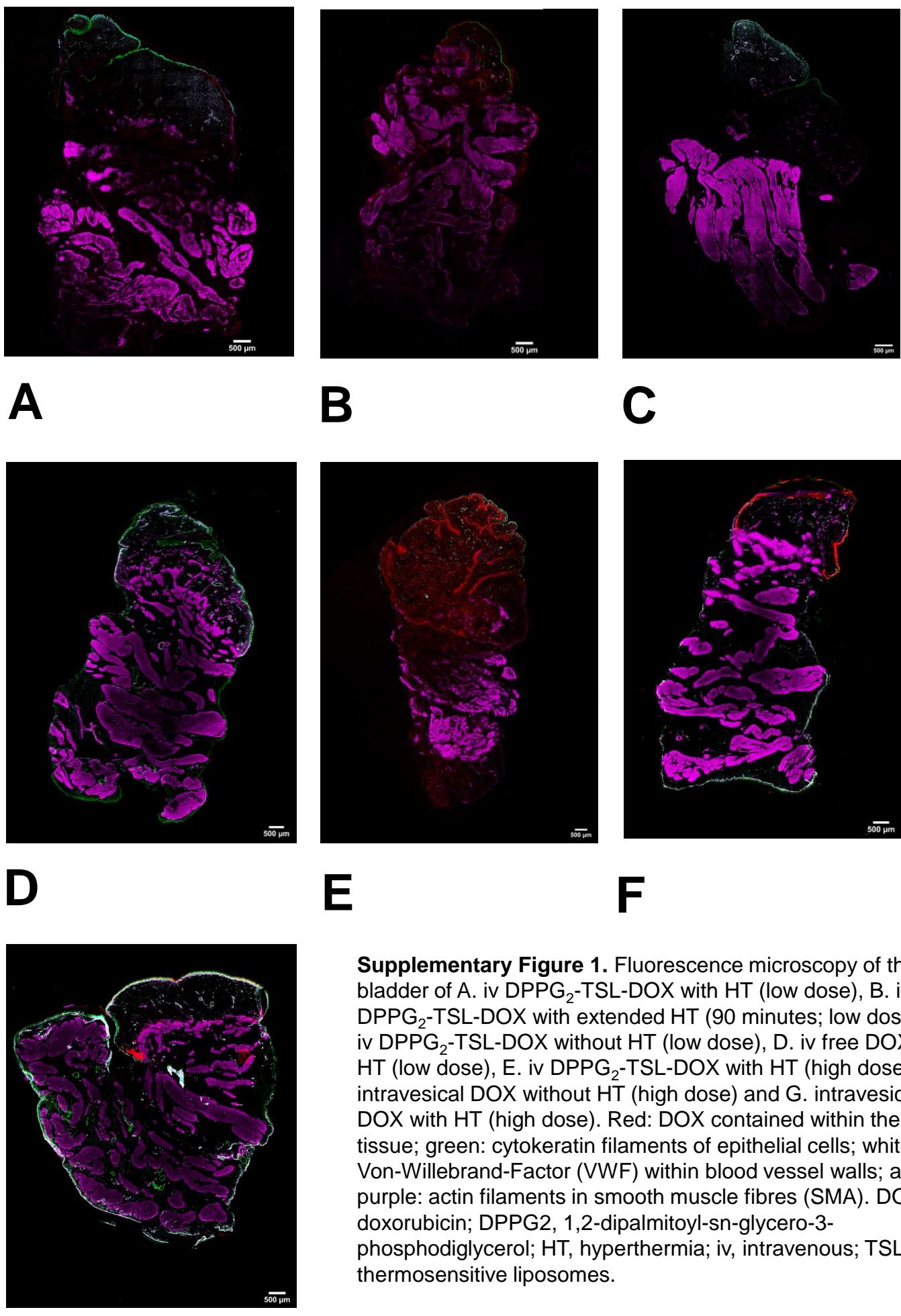


Supplementary Table 1 DOX concentrations per group, layer and sampling location

Group	left bladder neck			right bladder neck			left side wall			right side wall			left bladder dome			right bladder dome		
	M	D	S	M	D	S	M	D	S	M	D	S	M	D	S	M	D	S
DPPG ₂ -TSL-DOX (low dose) + HT	4.12	2.57	1.77	5.09	2.44	1.56	3.10	1.29	1.06	3.00	1.19	0.95	2.12	1.24	1.24	2.31	1.05	1.09
DPPG ₂ -TSL-DOX (low dose) + ext HT	±1.01	±0.70	±0.32	±1.43	±0.98	±0.68	±1.25	±0.28	±0.23	±1.02	±0.20	±0.07	0.65	±0.22	±0.21	±0.95	±0.18	±0.17
DPPG ₂ -TSL-DOX (low dose) - HT	2.45	1.03	1.03	1.51	0.90	1.05	3.68	1.52	1.13	3.00	1.24	1.32	3.67	1.31	1.26	3.13	1.66	1.44
DPPG ₂ -TSL-DOX (low dose) - HT	<0.20	<0.20	<0.20	<0.20	<0.20	0.10	<0.20*	<0.20	0.19	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20*	<0.20	0.15
iv free DOX (low dose) + HT	0.63	0.11	0.42	0.75	<0.20	0.28	0.46	<0.20	0.27	0.28	<0.20	0.36	0.68	0.13	0.38	0.50	0.10	0.30
DPPG ₂ -TSL-DOX (high dose) + HT	36.47	12.91	12.90	19.76	8.37	5.62	20.51	15.30	10.24	31.48	22.29	17.12	22.49	12.33	9.96	19.89	7.46	6.13
Intraves. DOX (high dose) + HT	±11.99	±2.62	±1.23	±3.94	±2.18	±0.72	±5.87	±5.92	±3.38	±16.94	±14.24	±9.11	±2.45	±2.87	±2.32	±5.53	±2.12	±2.51
Intraves. DOX (high dose) - HT	2.02	<0.20	0.29	5.91	0.31	0.43	5.42	0.16	<0.20	4.02	0.18	0.14	5.13	0.14	0.33	5.33	0.26	0.23
Intraves. DOX (high dose) - HT	±0.70	*	±0.24	±3.22	±0.13	±0.20	±3.25	±0.13	*	±1.19	±0.07	±0.12	±2.86	±0.11	±0.14	±3.33	±0.21	±0.18

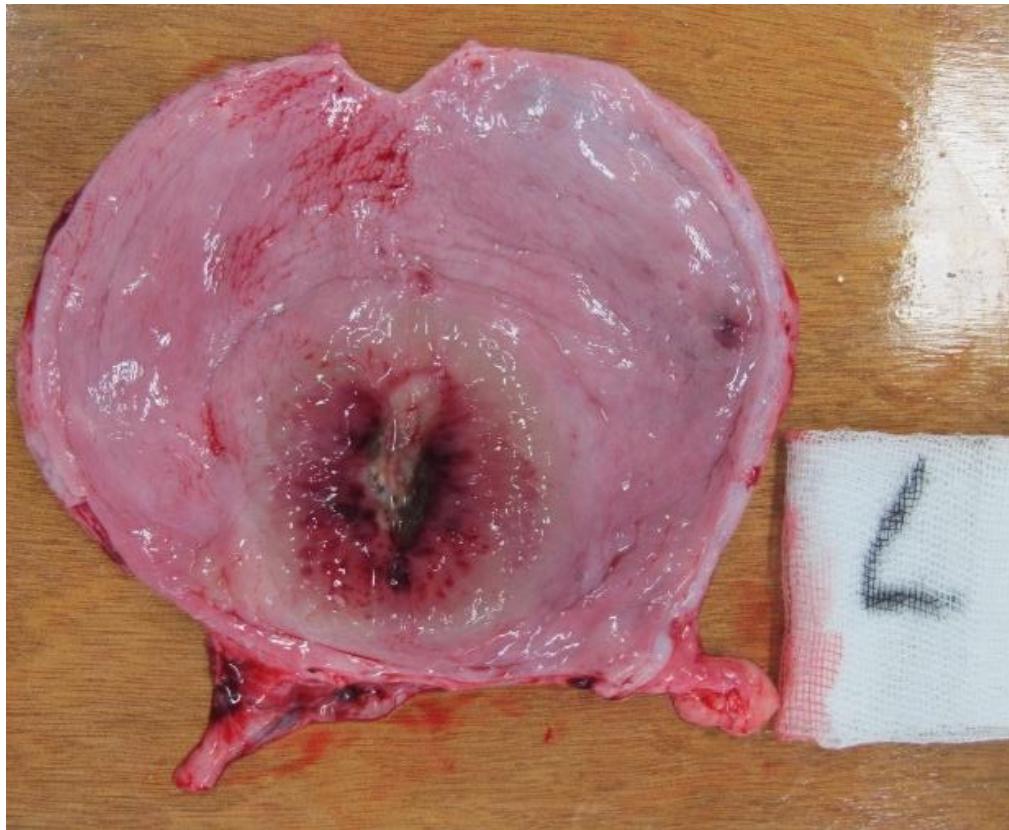
Notes: Concentrations are ± standard error of mean in ng/mg; * represents below limit of quantification.

Abbreviations: DOX, doxorubicin; DPPG₂, 1,2-dipalmitoyl-sn-glycero-3-phosphodiglycerol; HT, hyperthermia; intravesical, iv, intravenous; TSL, thermosensitive liposomes

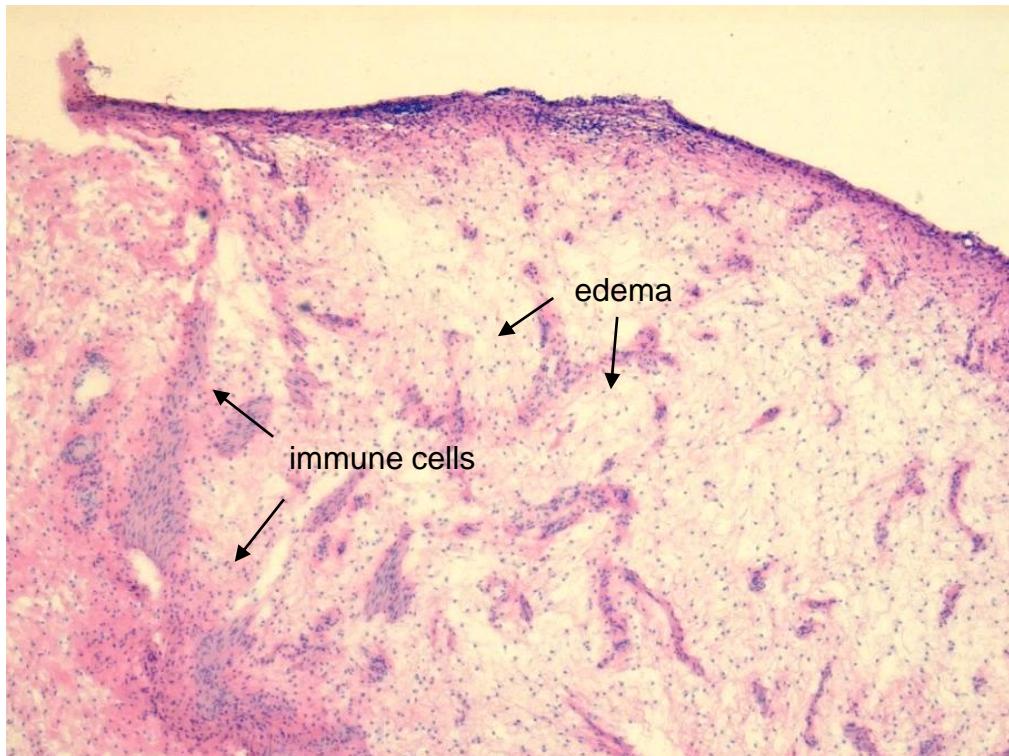


Supplementary Figure 1. Fluorescence microscopy of the bladder of A. iv DPPG₂-TSL-DOX with HT (low dose), B. iv DPPG₂-TSL-DOX with extended HT (90 minutes; low dose), C. iv DPPG₂-TSL-DOX without HT (low dose), D. iv free DOX with HT (low dose), E. iv DPPG₂-TSL-DOX with HT (high dose), F. intravesical DOX without HT (high dose) and G. intravesical DOX with HT (high dose). Red: DOX contained within the tissue; green: cytokeratin filaments of epithelial cells; white: Von-Willebrand-Factor (VWF) within blood vessel walls; and purple: actin filaments in smooth muscle fibres (SMA). DOX, doxorubicin; DPPG2, 1,2-dipalmitoyl-sn-glycero-3-phosphodiglycerol; HT, hyperthermia; iv, intravenous; TSL, thermosensitive liposomes.

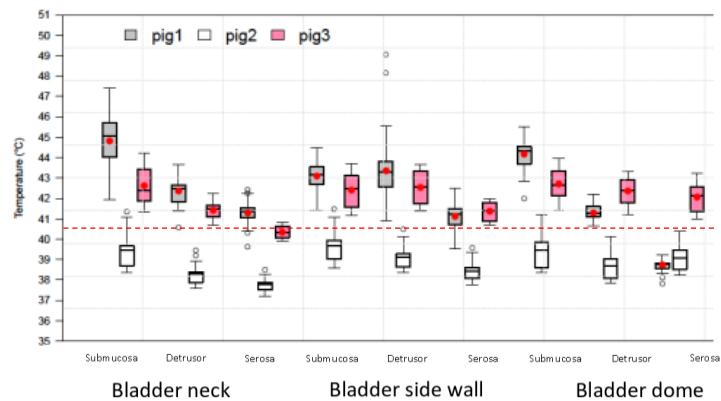
G



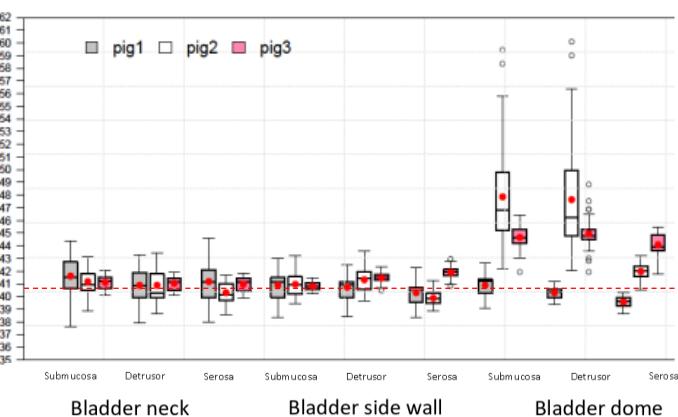
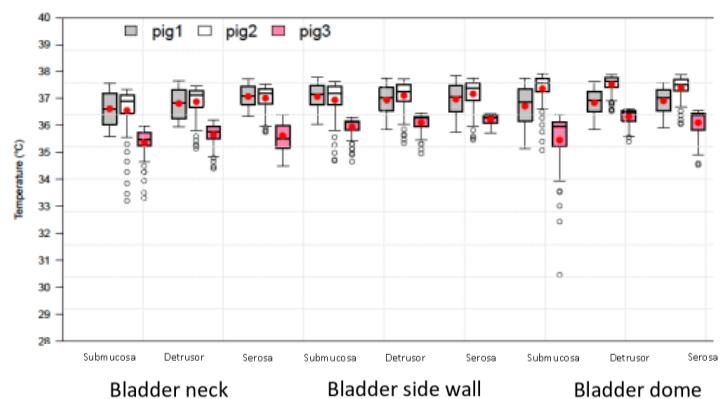
Supplementary Figure 2. Macroscopic photo of localized transmural thermal burn spot at the posterior side of the bladder (luminal view), including a hematoma and edema.



Supplementary Figure 3. Microscopic photo showing inflammatory reactions including immune cell infiltration and extensive submucosal edema (white vacancies).

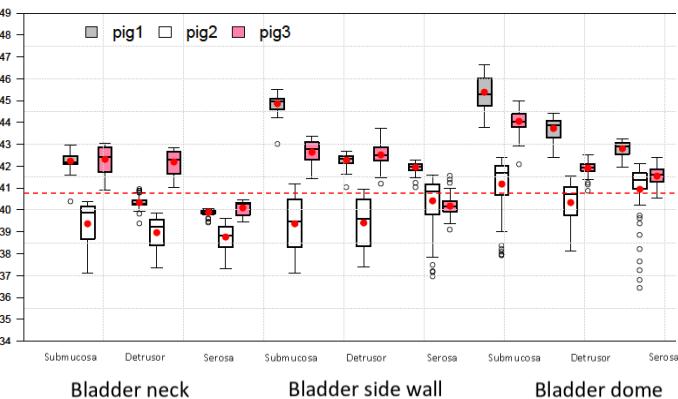
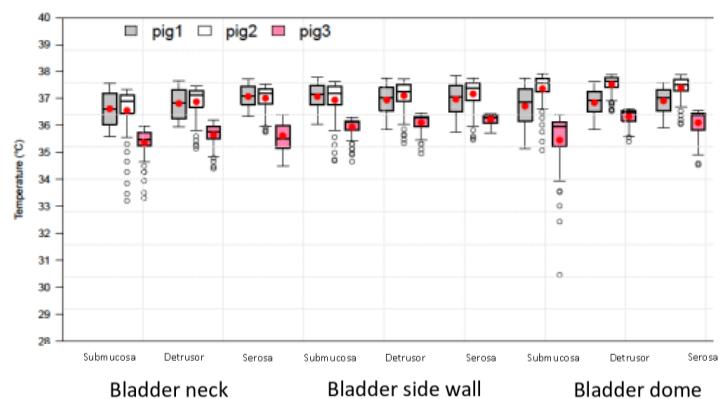


A



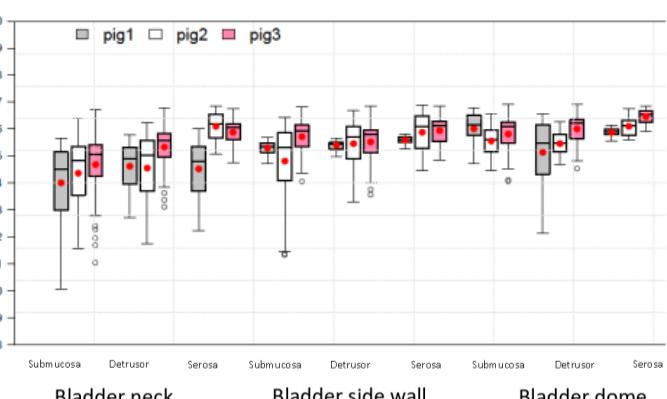
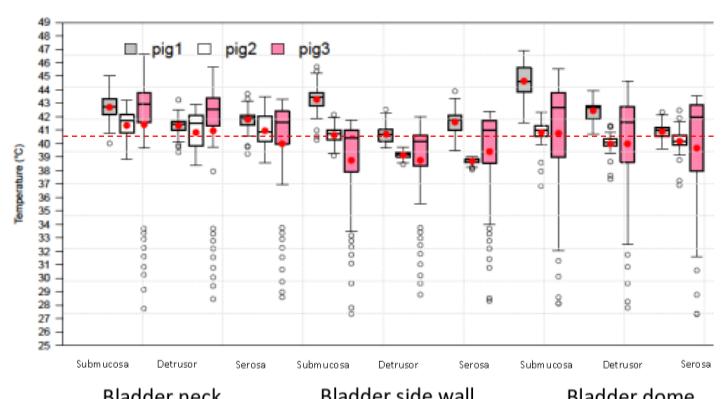
Bladder neck Bladder side wall Bladder dome

B



Bladder neck Bladder side wall Bladder dome

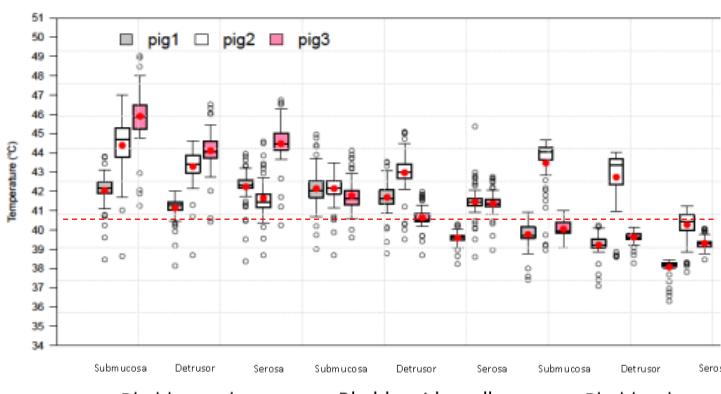
C



D

Bladder neck Bladder side wall Bladder dome

E



Bladder neck Bladder side wall Bladder dome

F

Supplementary Figure 4. Bladder wall temperatures of A. iv DPPG₂-TSL-DOX with HT (low dose), B. iv DPPG₂-TSL-DOX with extended HT (90 minutes; low dose), C. iv DPPG₂-TSL-DOX without HT (low dose), D. iv free DOX with HT (low dose), E. iv DPPG₂-TSL-DOX with HT (high dose), F. intravesical DOX without HT (high dose) and G. intravesical DOX with HT (high dose). Boxplots per pig and measurement location (mucosa, detrusor and serosa at bladder neck [first three], bladder side wall [middle three], and dome [last three]). Red dots represent the mean temperature and red dotted line a temperature of 40.5 °C. DOX, doxorubicin; DPPG2, 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol; HT, hyperthermia; iv, intravenous; TSL, thermosensitive liposomes.