Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information to

Multiparameter Urine Analysis for Quantitative Bladder Cancer Surveillance of Orthotopic Xenografted Mice

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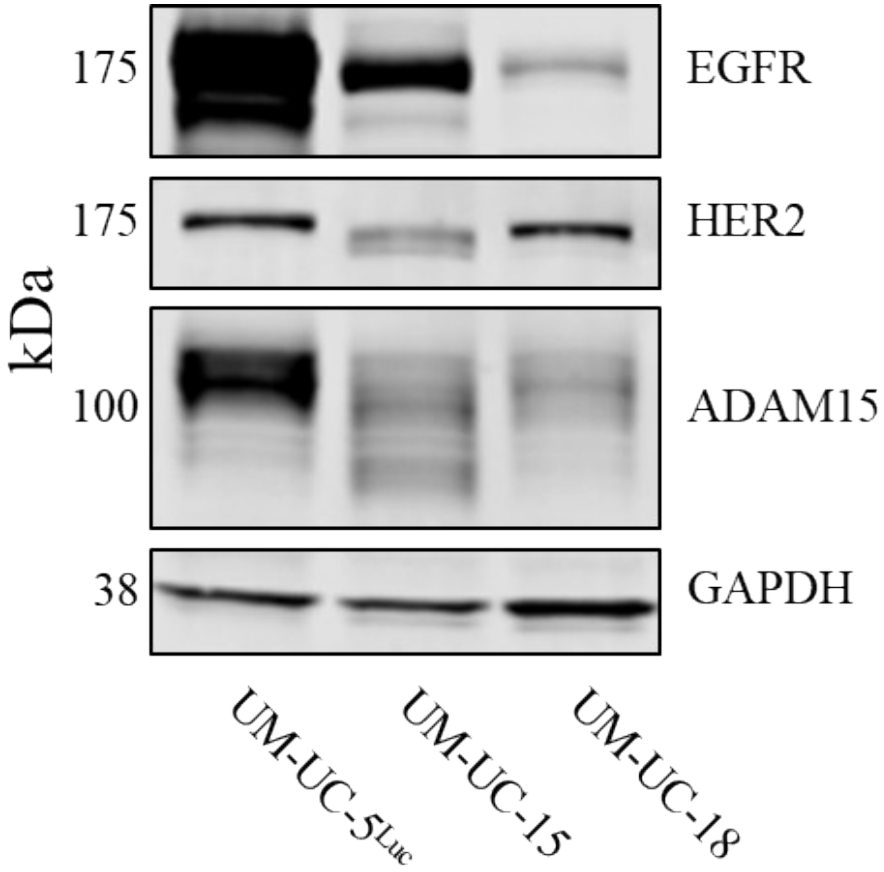

Figure S1. Bladder cancer cell line protein expression. Western blot showing each bladder cancer cell line's expression of the biomarkers of interest from 30 µg of whole cell lysate. Survivin was not detectable by Western blot with this much lysate (data not shown).

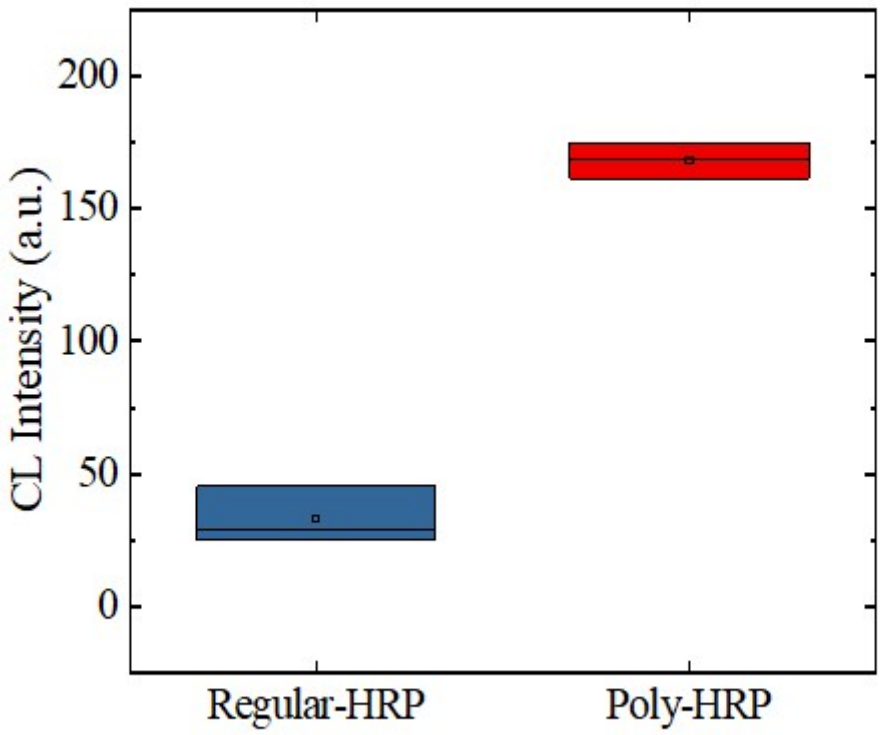

Figure S2. Signal amplification with streptavidin poly-HRP. A cartridge with 12 capillaries was used in this experiment. A common concentration of BSA-biotin (300 ng/mL) was first coated on the first six capillaries, whereas the remaining six capillaries were incubated with $1 \times PBS$ for background noise measurements. Then a standard two-steps blocking was performed before adding streptavidin regular-HRP or streptavidin poly-HRP. Four groups of results were obtained in this experiment (BSA-biotin/regular-HRP, BSA-biotin/poly-HRP, PBS/regular-HRP and PBS/poly-HRP), each of which contains three capillaries. As the results show, the averaged chemiluminescent signal intensity from the BSA-biotin/regular-HRP group was 33.2 and the averaged chemiluminescent intensity from the BSA-biotin/poly-HRP was 168.4. Both the regular-HRP and poly-HRP groups were observed to have background noise levels lower than 0.1. Therefore, the use of streptavidin poly-HRP can enhance the absolute signal and the signal-to-noise ratio 5-fold compared to those with the regular streptavidin HRP.

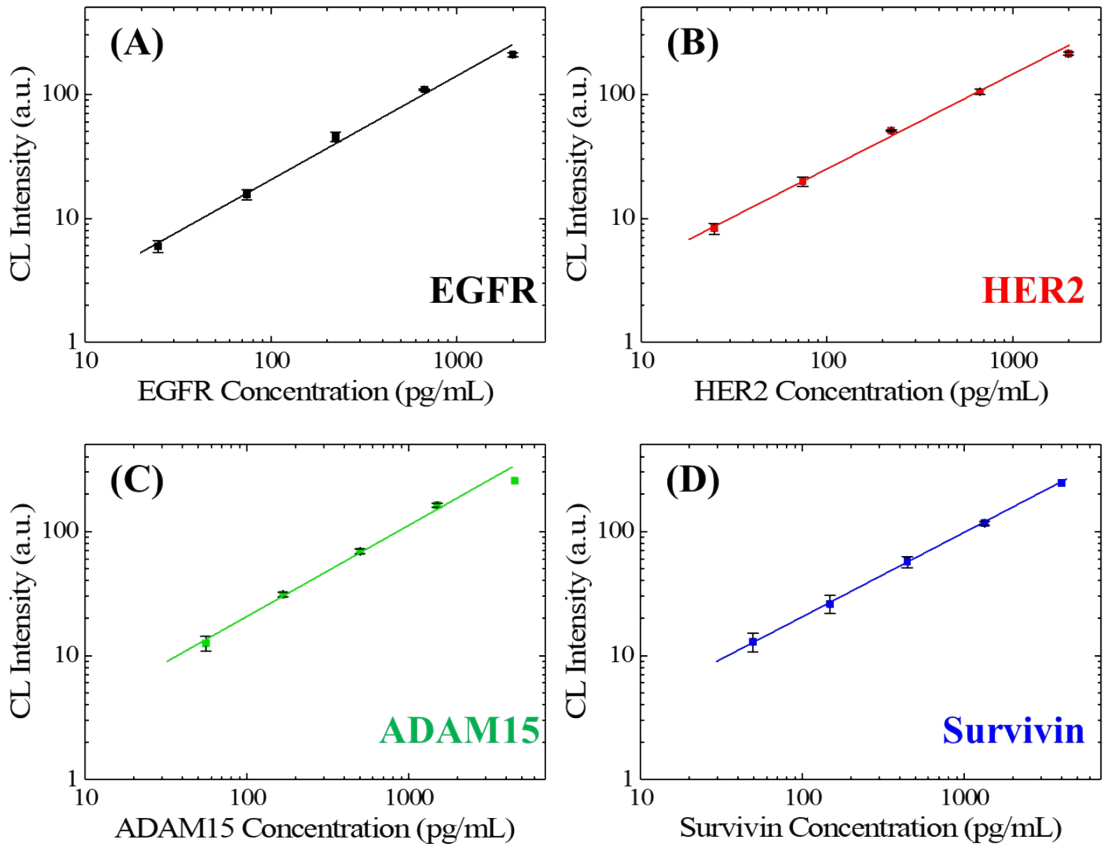

Figure S3. Calibration data points for the linear ranges (in the log-log scale) of the four biomarkers. The solid lines are the linear fits in the log-log scale. The error bars represent the intra-assay variances that were obtained from duplicated measurements. All intra-assay variances were close to or smaller than 10%.

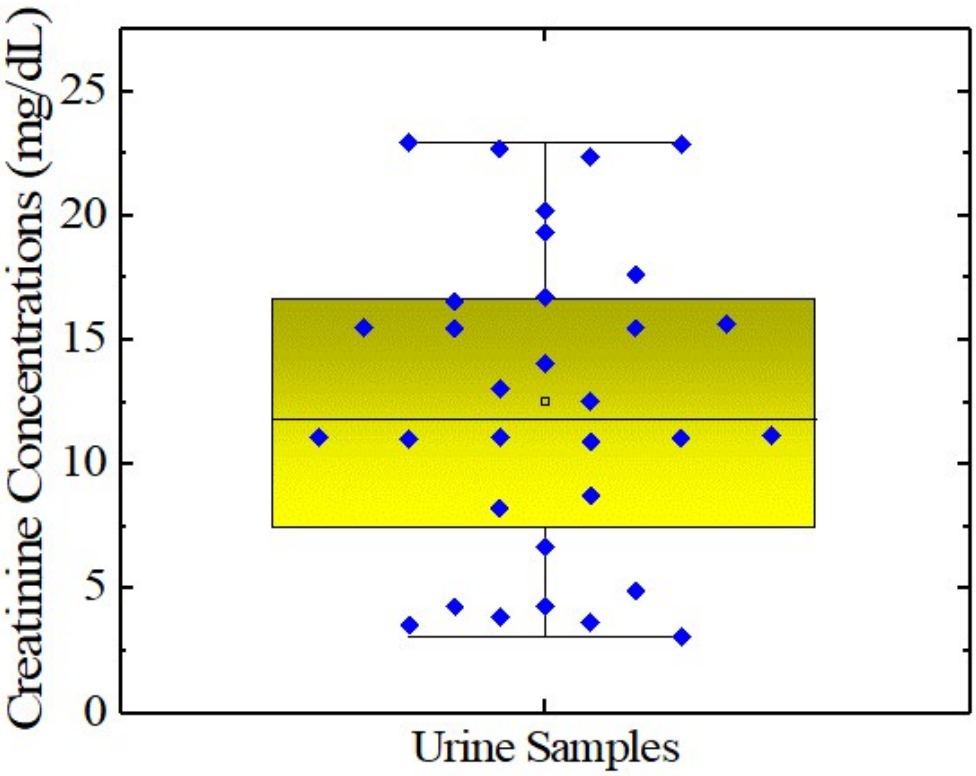

Figure S4. Box plot for the creatinine concentration measurements in the urine samples. The creatinine concentrations for most of the samples were between 4-23 mg/dL, which is narrower than an order of magnitude. The samples with lower creatinine concentrations were mostly collected from the mice at later stages (the 3rd or 4th week).

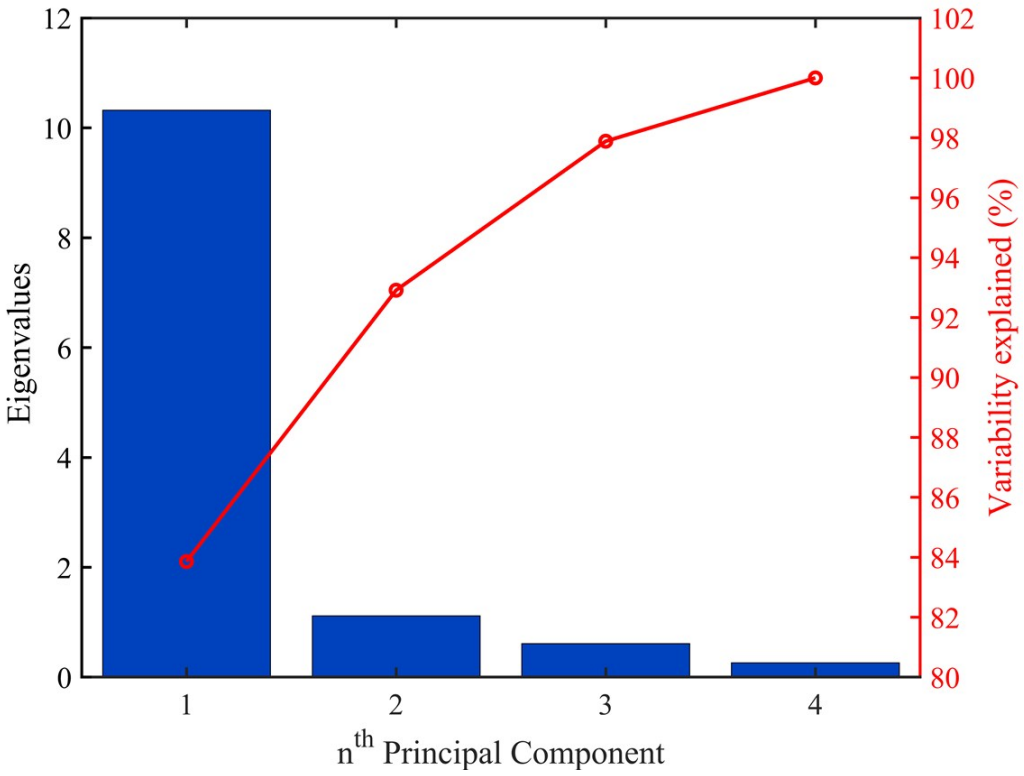

Figure S5. The eigenvalues for the four PC scores are 10.32, 1.11, 0.61, and 0.23, respectively. Which means the four PC scores can account for 84.1 %, 9.0%, 5.0%, and 1.9% of the variabilities, respectively. Based on the eigenvalue, 93% variability was accounted for with the first two PCs.

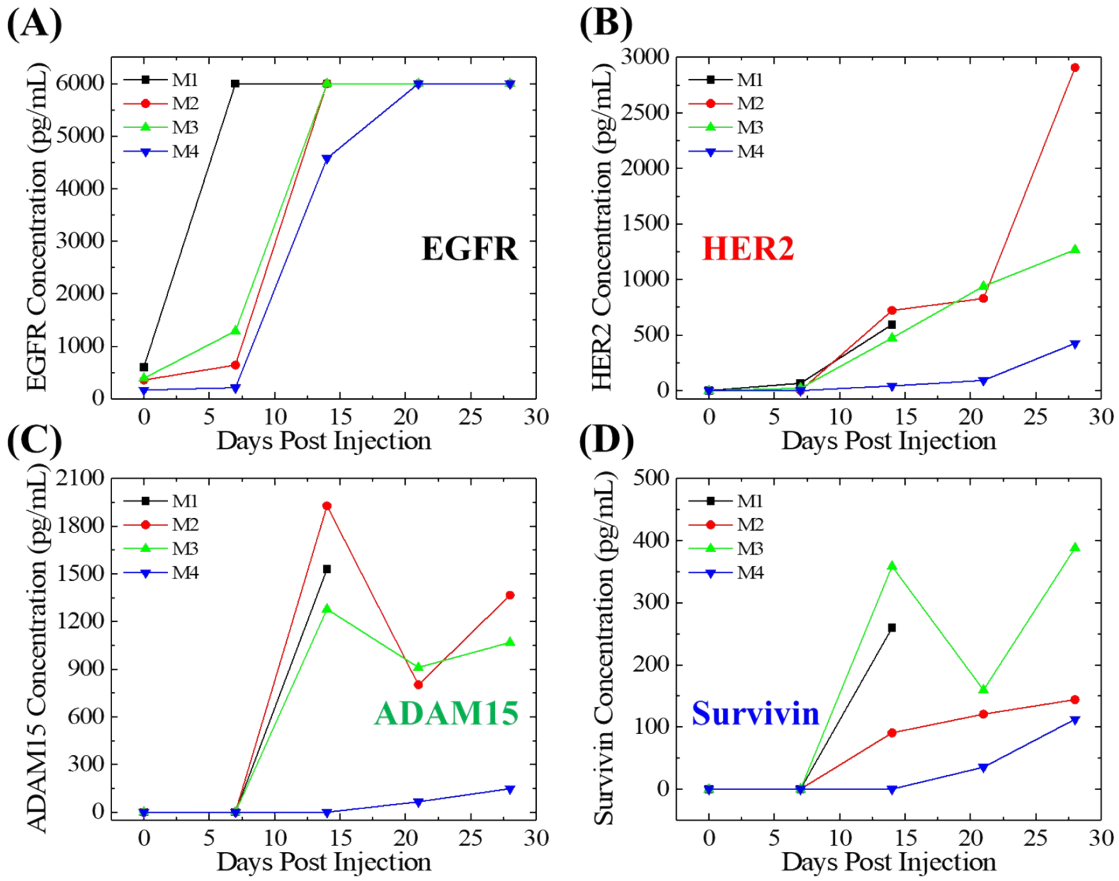

Figure. S6. ELISA readings for the four biomarkers in the "medium number" group (1.0 million of UMUC cells). The trends were generally similar to the "low number" group, with higher absolute concentrations.

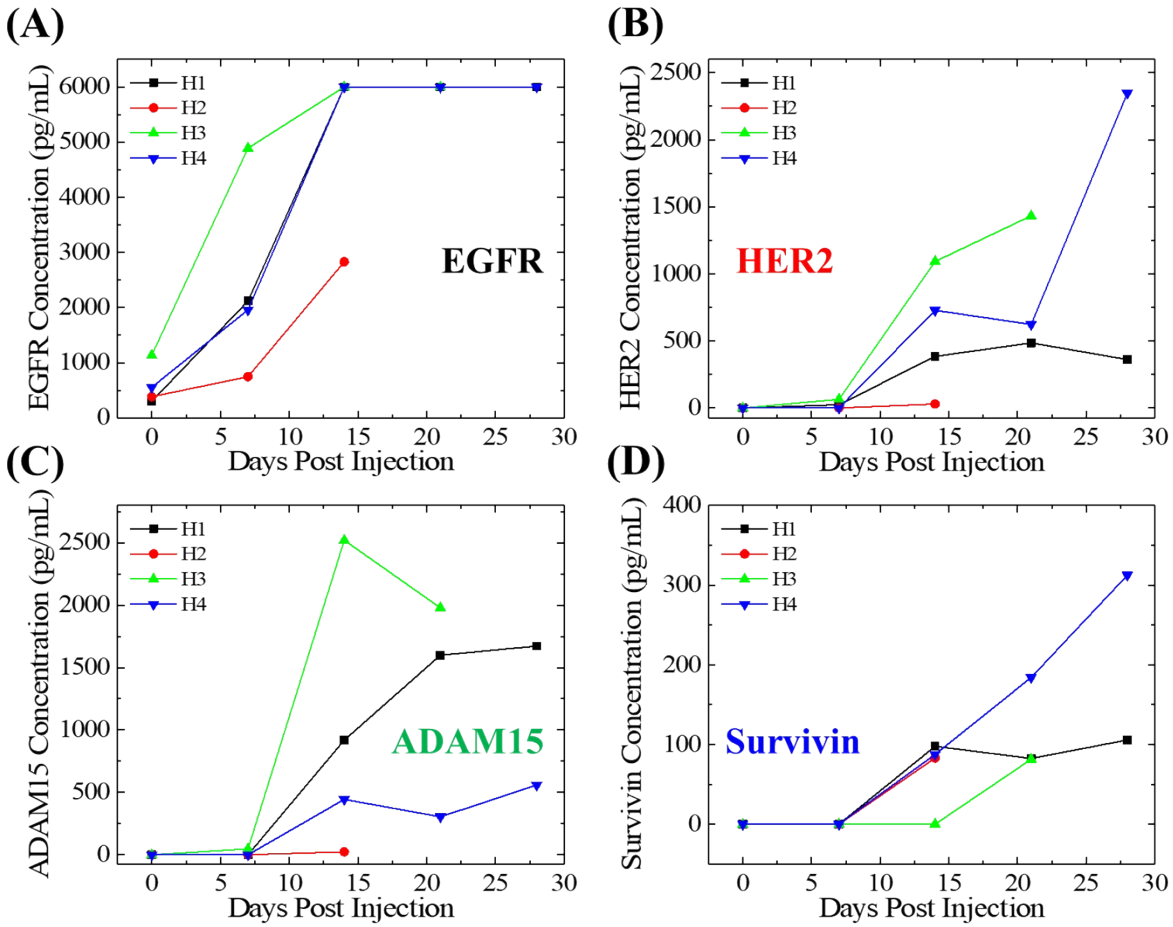

Figure S7. ELISA readings for the four biomarkers in the "high number" group (1.5 million of UMUC cells). The trends were generally similar to the "low number" and the "medium number" groups.

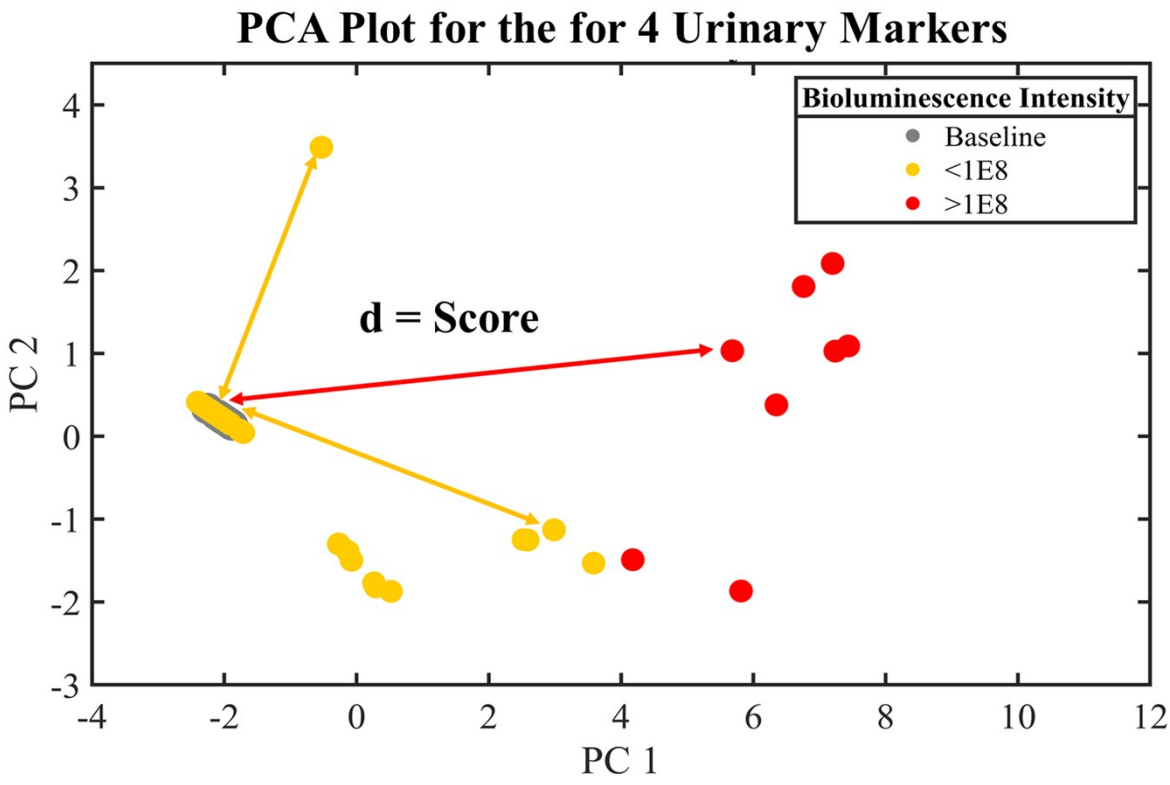

Figure S8. The "tumor growth score" is calculated as the distance from the point in the PCA plot and the averaged baseline point centered around (-2, 0.2).

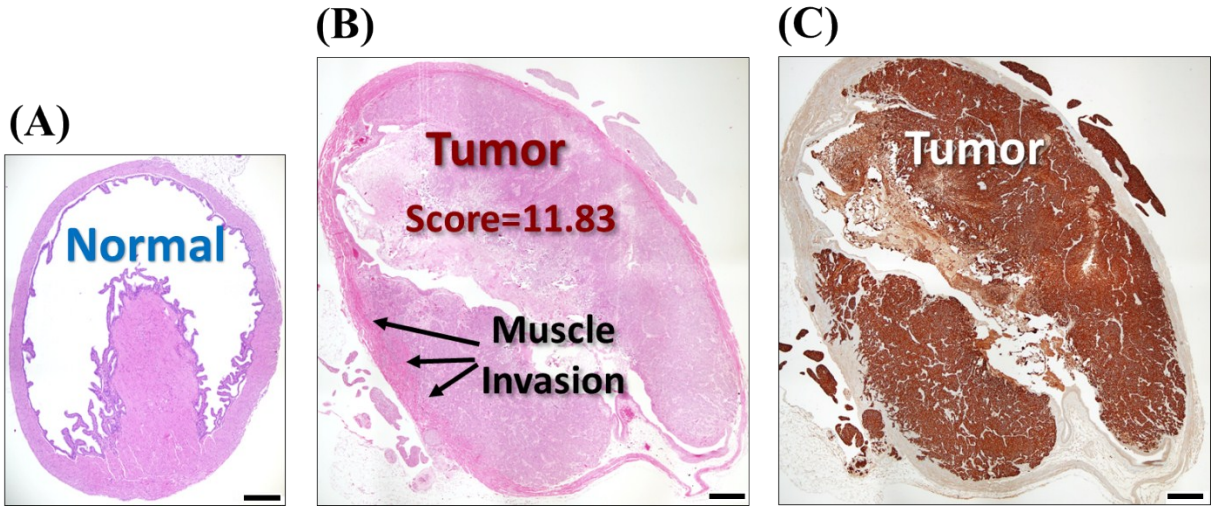

Figure S9. Histology slides for a normal mouse bladder and a mouse bladder filled with human tumor (from mouse M2). (A) H&E staining for the cross-section of a normal mouse bladder without tumor. (B) H&E staining for the cross section of mouse M2's bladder. Muscle invasion can be found on the left edge of the cross-sectional image, as marked by the arrows. The corresponding tumor growth score is 11.83. (C) EGFR immunohistochemistry staining for the cross section of mouse M2's bladder. The bladder lumen was almost filled with tumor and the tumor appeared to be very thick. In addition, the bladder with tumor has significantly larger size than the normal bladder. All scale bars: 500 μm.

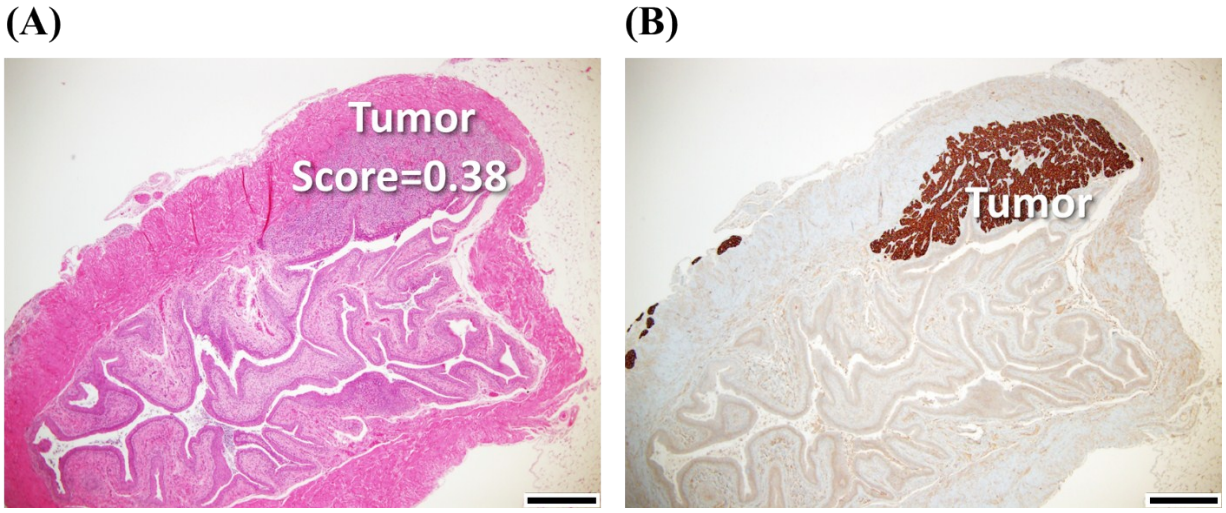

Figure S10. Histology slides for a mouse bladder with tumor, after three weeks of Dacomitinib treatment. (A) H&E staining for the cross-section of mouse D2's bladder. The corresponding tumor growth score for this bladder is 0.38. (C) EGFR immunohistochemistry staining for the cross section of mouse D2's bladder. The tumor appeared to be small and loose.

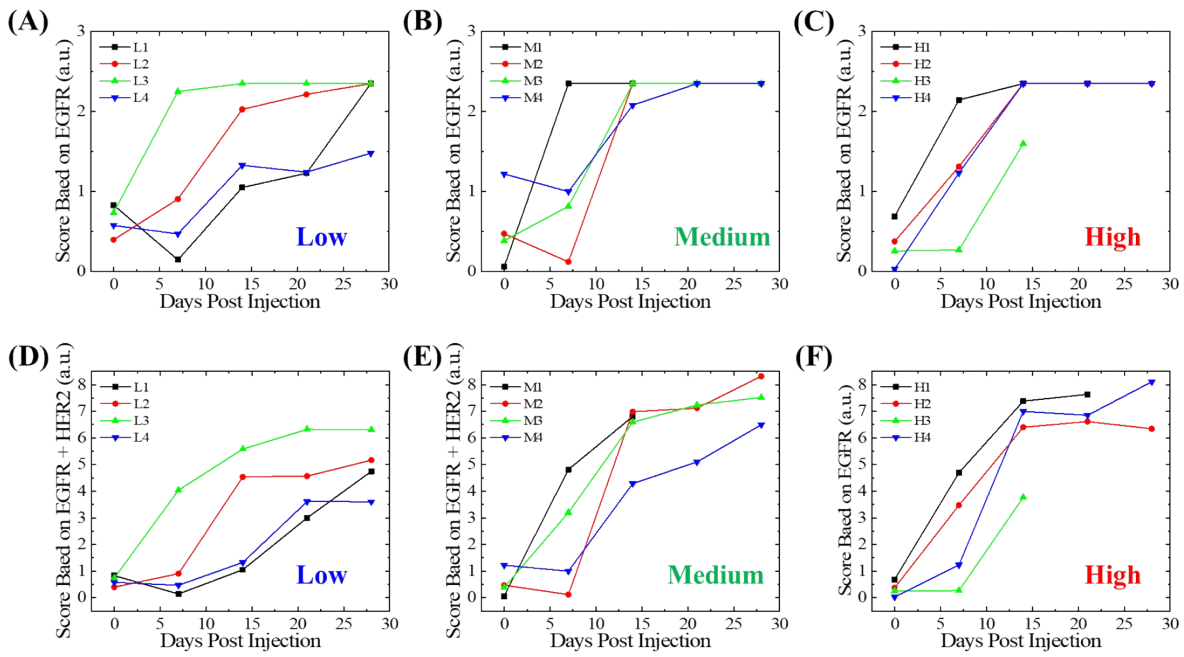

Figure S11. Tumor growth scores based on urinary EGFR only ((A)-(C)) and EGFR+HER2 ((D)-(F)). (A)-(C). The tumor growth scores based on urinary EGFR concentrations. Each point represents the absolute distance (*i.e.*, values are always positive) between each measurement and the grand averaged background (averaged among all mice). The background variance at Day 0 is obvious for all groups. (D)-(F). The tumor growth scores based on urinary EGFR and HER2 concentrations. The noises on Day 0 are significantly improved but still not as good as the four-marker model.

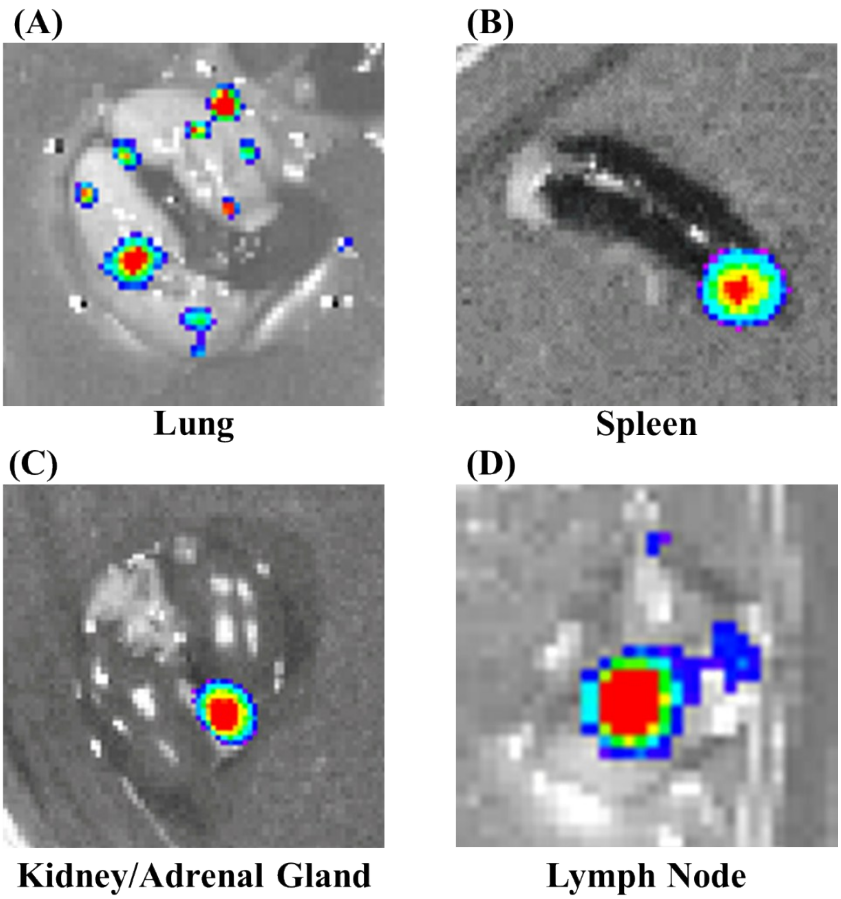

Figure S12. Bioluminescence images for metastasis at distant organs. Human bladder cancer cells were observed in lung, spleen, kidney/adrenal gland and lymph nodes. Subfigure (A) was collected from mouse L2's lung; (B) was collected from mouse M3's spleen. (C) was collected from mouse M4's kidney/adrenal gland and (D) was collected from mouse L1's lymph node. The subfigures were not on the same scale.