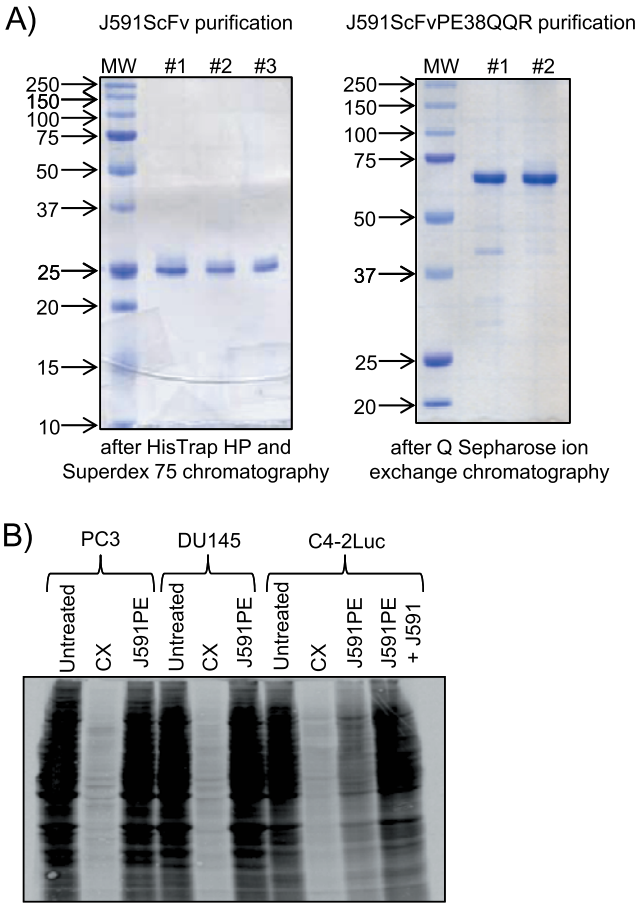

Figure W1. Purification and testing of J591PE. (A) Purification of J591scFv and J591scFvPE38QQR (J591PE) was evaluated using Coomassie staining after SDS-PAGE on 10% gels. Representative pictures are reported. The most purified batches were also filtered using a 0.22-μm syringe filter, evaluated for activity (see Figure 1*D*), aliquoted, and stored in PBS at −20°C for subsequent experiments. (B) J591PE selectively inhibits protein synthesis in PSMA-positive C4-2Luc cells. PSMA-negative PC3, DU145, and PSMA-positive C4-2Luc cells were treated with 100 μg/ml cycloheximide (CX) or 1 μg/ml J591PE for 5 hours and labeled with S^{35}cystein-methionine for 1 hour. C4-2Luc cells were also preincubated with 10 μg/ml J591 antibody for 1 hour before adding J591PE. Protein lysates (25 μg) were separated by SDS-PAGE, transferred to nitrocellulose membranes, and exposed to PhosphorImager screen for 5 days.

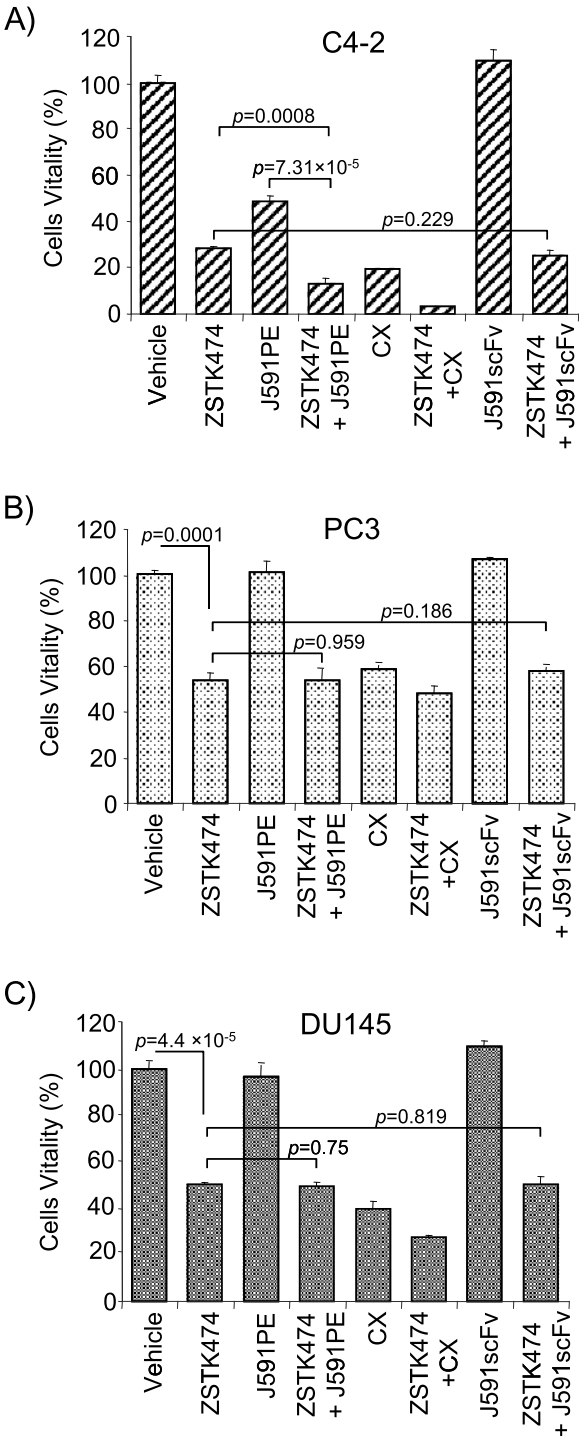

Figure W2. Analysis of long-term viability in prostate cancer cells treated with ZSTK474, J591PE, or the combination. Combination of 2 μg/ml ZSTK474 with 1 μg/ml J591PE selectively and significantly reduces viability in PSMA-positive C4-2 cells (A) at 48 hours, when compared with controls or PSMA-negative PC3 (B) and DU145 (C) cells (CX, 100 μg/ml cycloheximide; J591scFv was used at a concentration of 1 μg/ml). Cell viability was analyzed by MTT assay.

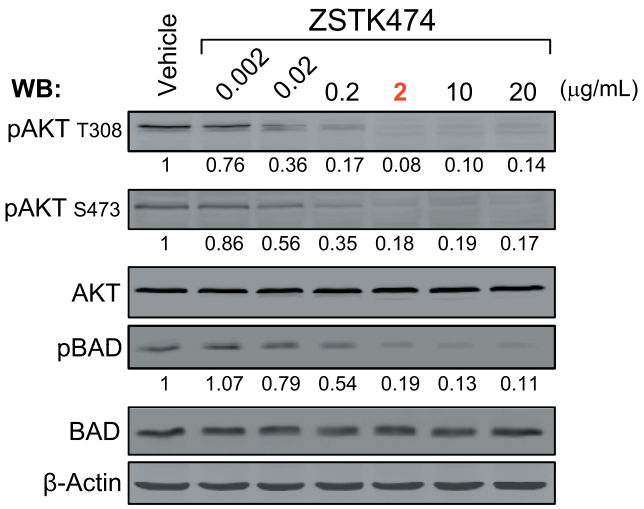

Figure W3. Inhibition of PI3K by ZSTK474. Titration of ZSTK474 in C4-2 cells showed that maximal inhibition of PI3K is exerted at 2 μg/ml (5 μM), by monitoring the p-AKT levels at T^{308} and S^{473}, at 6 hours. A representative Western blot panel showing also the phosphorylation status of BAD (S^{112}), a downstream target of AKT (vehicle, DMSO), is reported.

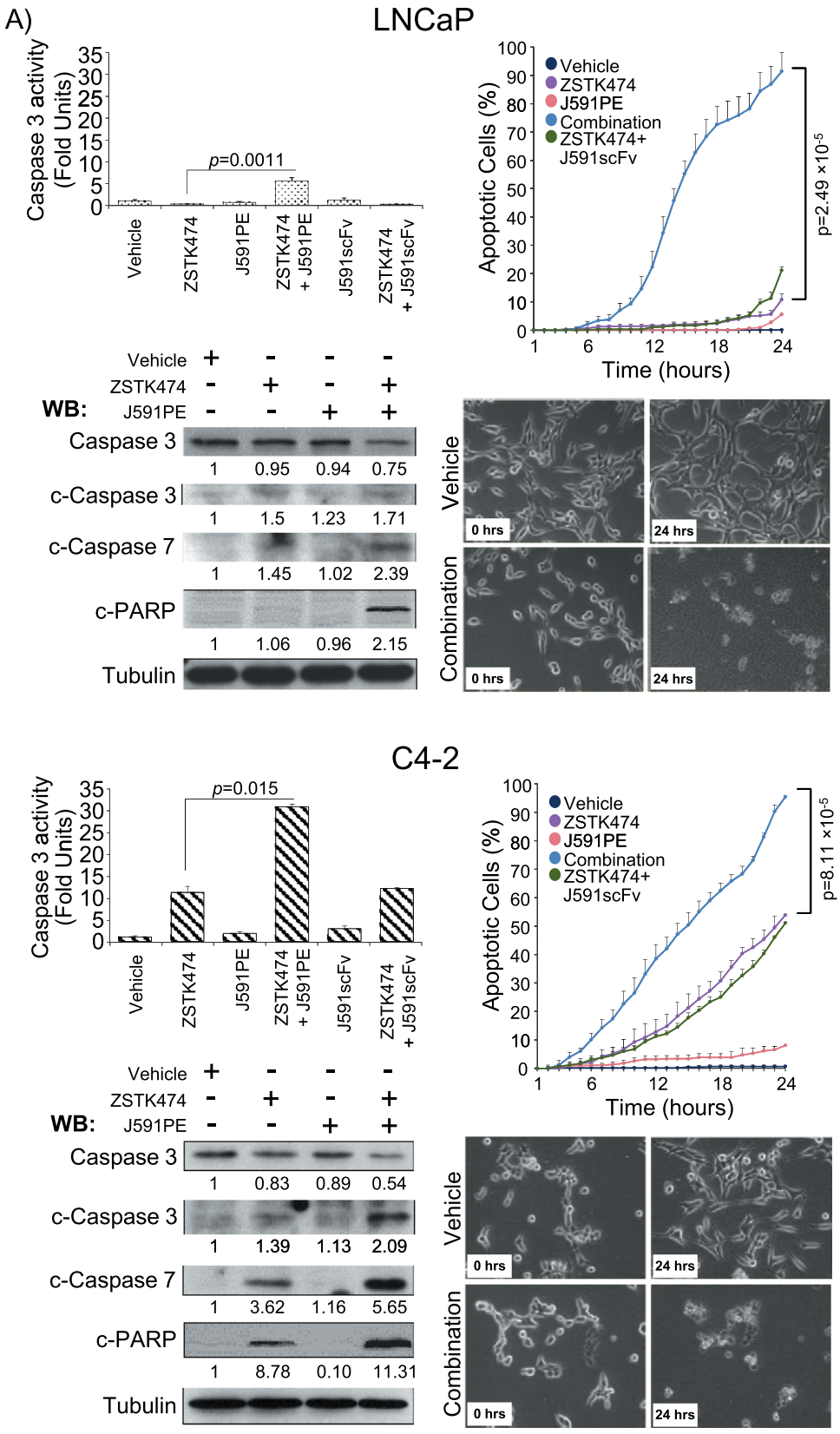

Figure W4. Induction of apoptosis in prostate cancer cells by combination of ZSTK474 and J591PE. (A) Combination of ZSTK474 and J591PE promotes apoptosis at 6 hours in LNCaP and C4-2 cells, as suggested by the analysis of caspase 3 activity, cleavage of effector caspases 3 and 7, cleavage of PARP, and analysis of cell morphology by time-lapse video recording. (B) Combination of ZSTK474 and J591PE does not increase apoptosis in BT549 cells, when compared to single-agent treatments. Results are expressed as means ± SEM, $n = 2$ (caspase 3 assay) and $n = 4$ (time-lapse video microscopy). Representative Western blots and time-lapse video microscopy frames (original magnification, ×20) are shown (vehicle, DMSO; combination, ZSTK474 + J591PE).

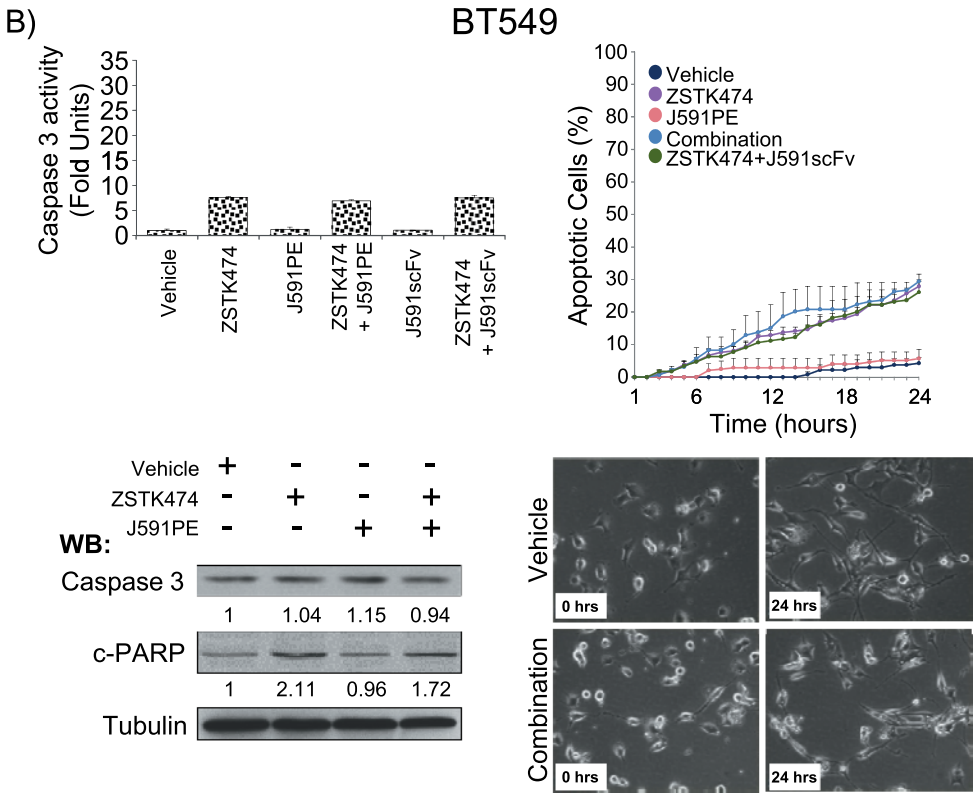

Figure W4. (*continued*).

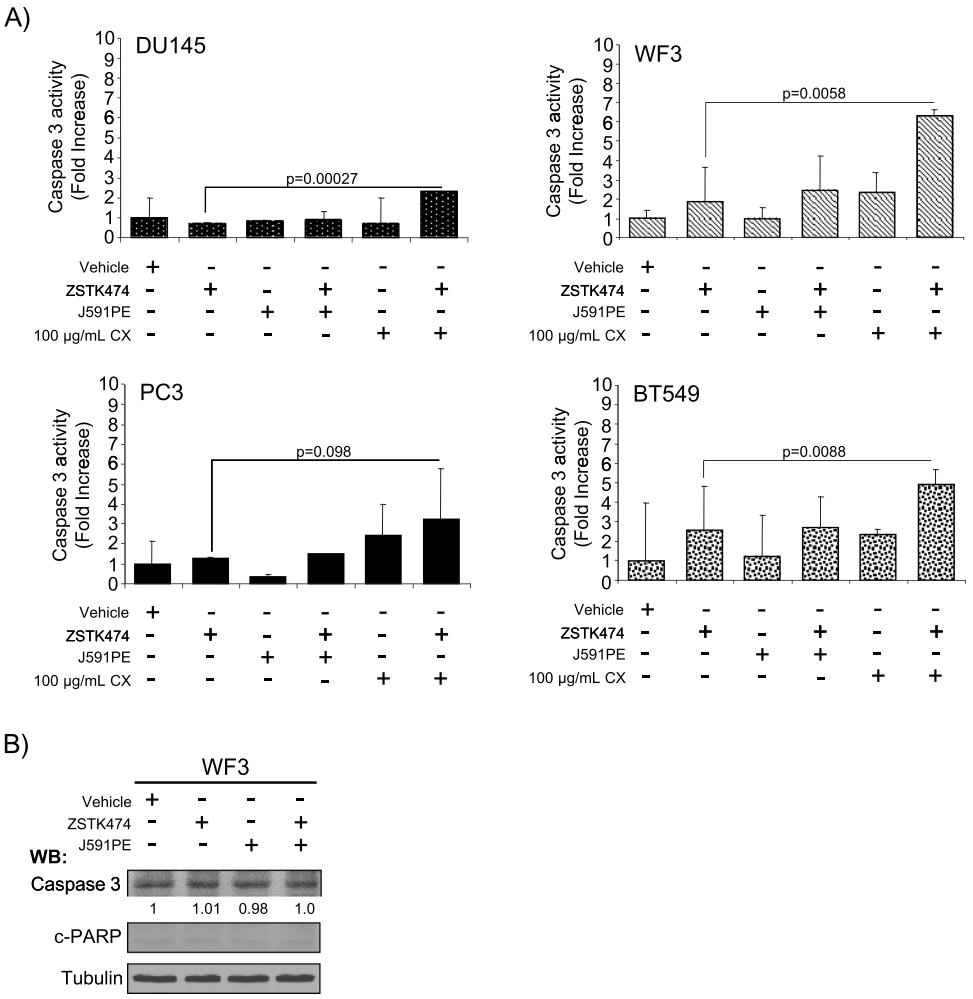

Figure W5. Combination of ZSTK474 and J591PE does not increase caspase 3 activity or PARP cleavage in PSMA-negative WF3, PC3, BT549, and DU145 cells. (A) Caspase assays in DU145, WF3, PC3, and BT549 cells treated with ZSTK474, J591PE, or the combination of ZSTK474 and J591PE. As a positive control for protein synthesis inhibition, cycloheximide (CX) was used, which significantly increased caspase 3 activity when combined with ZSTK474. (B) Western blot analysis of full-length caspase 3 and cleaved PARP in WF3 cells treated with ZSTK474 alone or in combination with J591PE. (C) Western blot analysis of DU145 cells treated with ZSTK474 alone or in combination with J591PE. Cycloheximide (CX) was used as a positive control for protein synthesis inhibition. PI3K inhibition was followed by monitoring p-AKT (T^{308} and S^{473}) and p-BAD (S^{112}) levels, downstream targets of PI3K. Insulin-like growth factor 1 (IGF-1; 500 ng/ml) treatment of DU145 cells was used as a positive control for PI3K activation. The arrow pointed at the cleaved caspase 7 fragment and its quantification. Treatments were normalized to their respective controls (vehicle, DMSO; combination, ZSTK474 + J591PE; CX, cycloheximide). Results are expressed as means ± SEM, $n = 2$. Representative Western blots are shown.

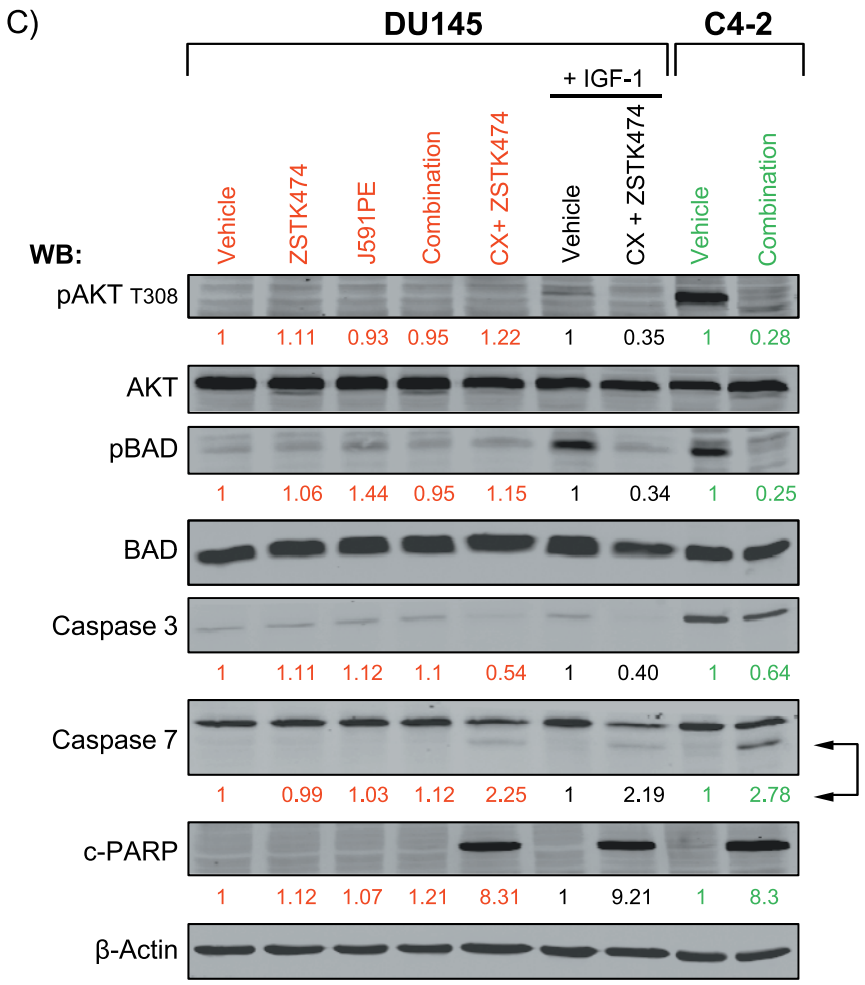

Figure W5. (*continued*).

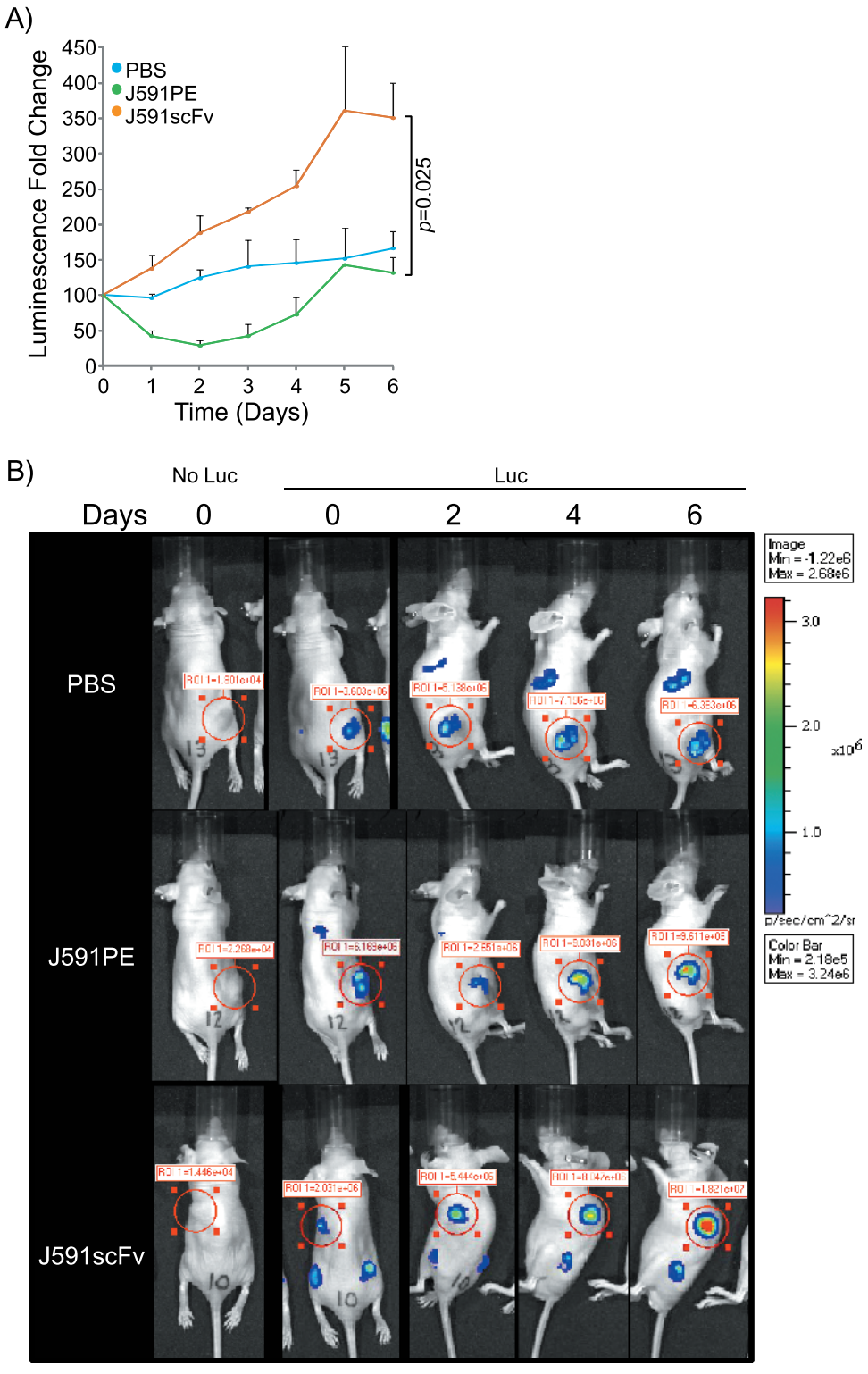

Figure W6. (A) Dynamics of luminescence of C4-2Luc tumors after a single local injection of PBS, J591PE (2.5 mg/kg), or J591scFv (2.5 mg/kg). Luminescence was monitored for 6 days by optical imaging and expressed as fold change compared to luminescence at day 0. Results are expressed as means ± SEM, $n = 3$ (J591scFv), $n = 4$ (PBS), and $n = 10$ (J591PE). (B) Representative images of nude mice recorded on IVIS 100 luminescent imaging station showing ROI, 15 minutes after i.p. luciferin injection. (C) Representative images of nude mice reported in Figure 6*C* and in B showing luminescence background inside ROI beside tumors treated with control vehicles (DMSO or PBS), ZSTK474, J591PE, or J591scFv, 15 minutes after i.p. injection of luciferin.

C)

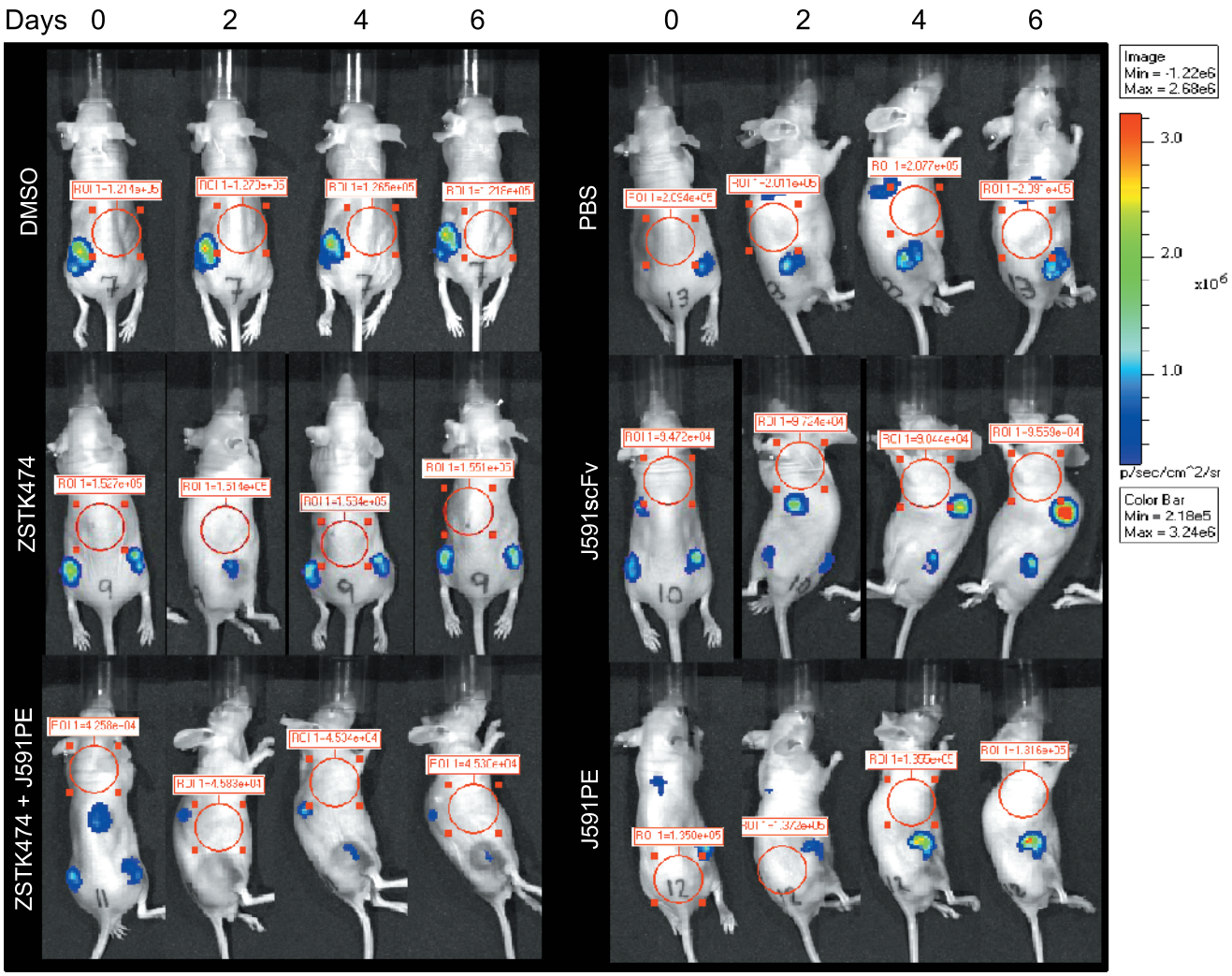

Figure W6. (*continued*).