## Supplemental Material

# PRL3-zumab, A First-in-Class Humanized Antibody for Cancer Therapy

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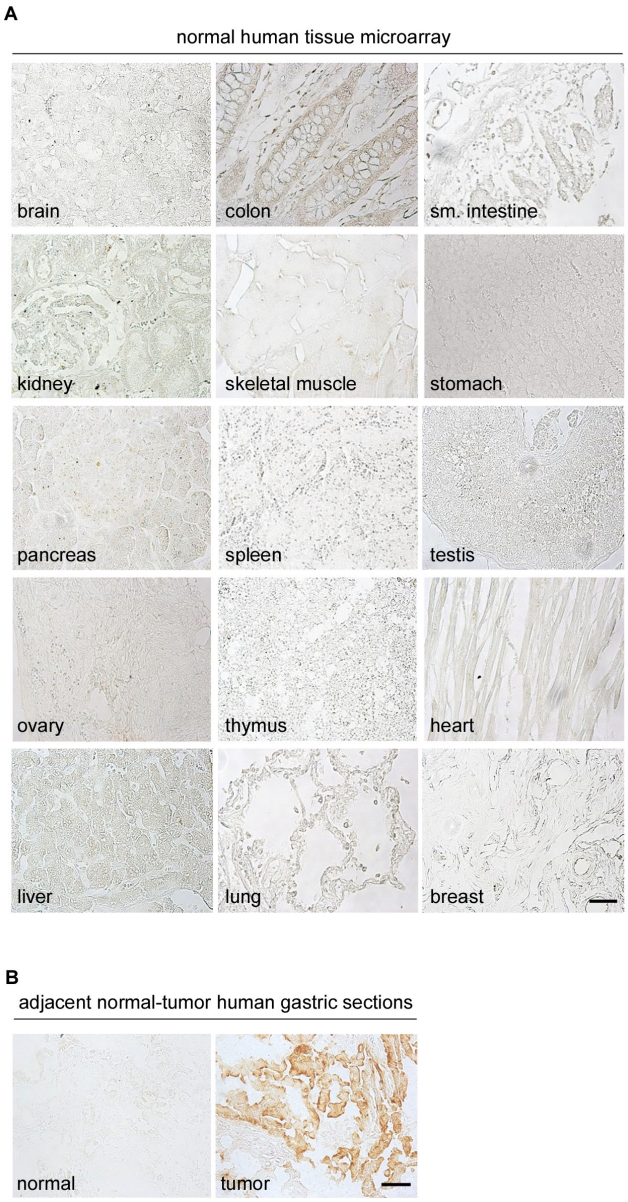
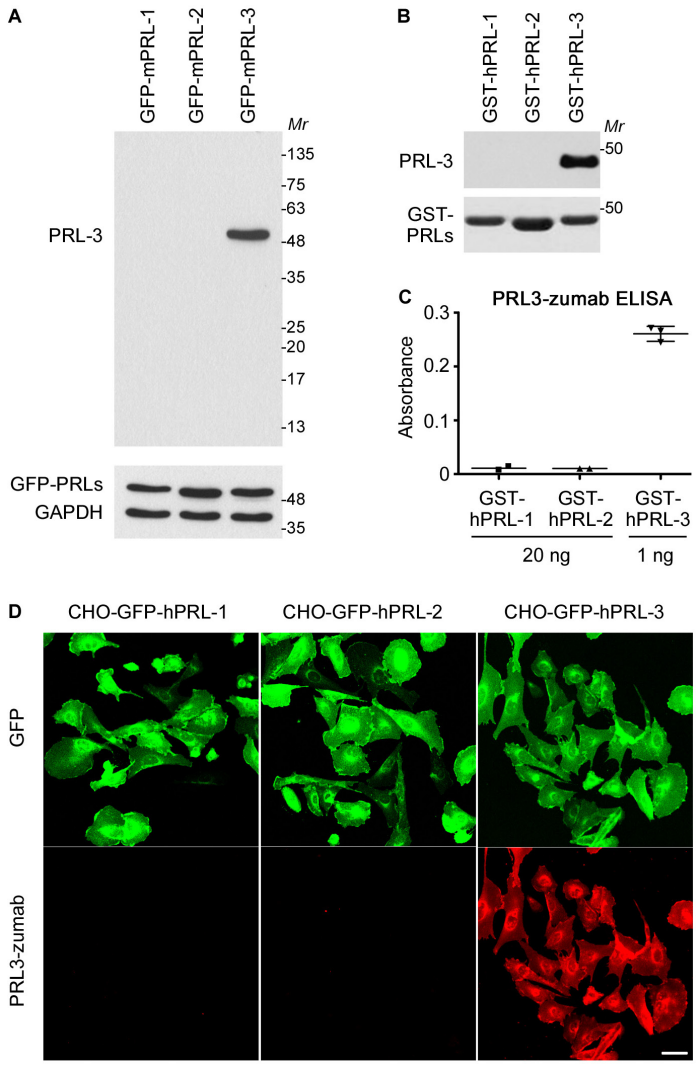
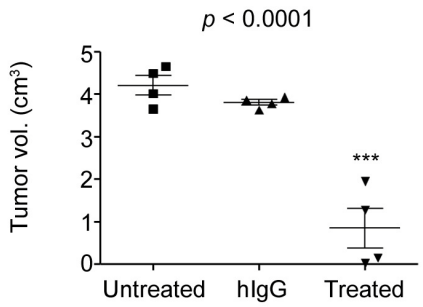


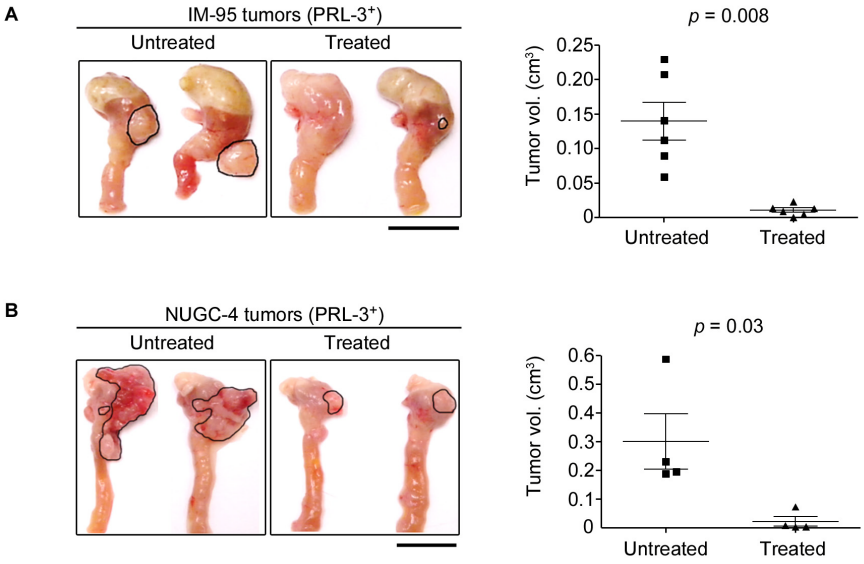

**Supplemental Figure 1.** PRL-3 is not expressed in most normal adult human tissues, but strongly expressed in human gastric tumors. **(A)** Multiple normal human tissues from various organs were analyzed by immunohistochemistry (IHC) for PRL-3 protein. **(B)** By IHC, PRL-3 protein was not detected in an adjacent normal gastric tissue section, but was strongly detected in gastric tumor section from the same patient. *Bar*, 50μm.



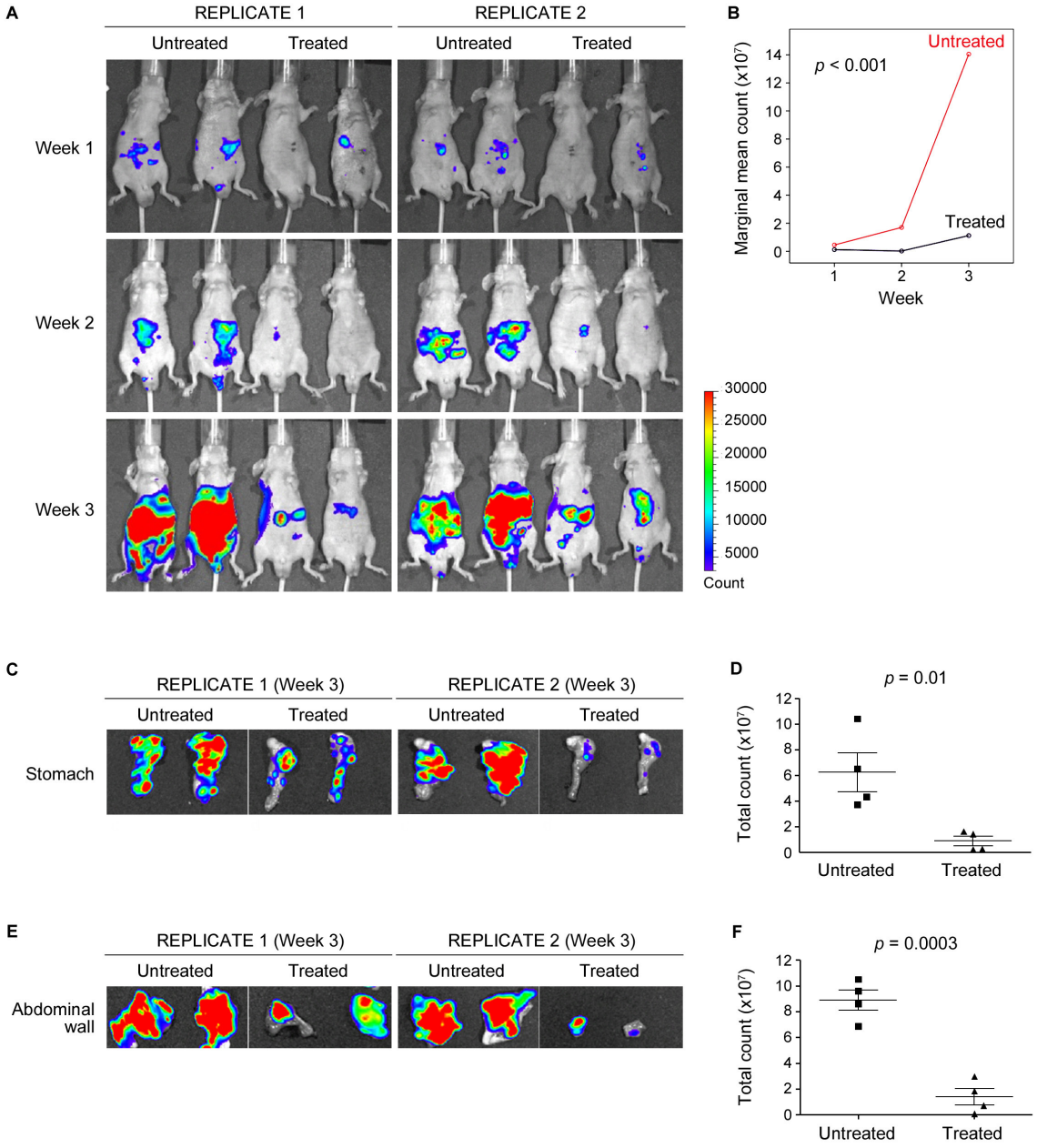

**Supplemental Figure 2.** PRL3-zumab specifically binds to both murine and human PRL-3, but not to their homologues PRL-1 or PRL-2. **(A)** Western blotting of Chinese Hamster ovary (CHO) cells overexpressing GFP-tagged murine isoforms of PRL-1 (GFP-mPRL-1), PRL-2 (GFP-mPRL-2), or PRL-3 (GFP-mPRL-3). Blots were probed with PRL3-zumab (upper panel), or anti-GFP and anti-GAPDH antibodies concurrently (lower panel). **(B)** Western blotting of 1 ng of GST-tagged recombinant human isoforms of PRL-1 (GST-hPRL-1), PRL-2 (GST-hPRL-2), or PRL-3 (GST-hPRL-3). Blots were probed with PRL3-zumab (upper panel) or anti-GST antibodies (lower panel). **(C)** ELISA analysis of PRL3-zumab specific binding to recombinant GST-hPRLs. PRL3-zumab readily binds GST-hPRL-3, but not 20-fold higher amounts of GST-hPRL-1 or GST-hPRL-2. $n = 3$ per analysis; $p < 0.001$, one-way ANOVA; data representing mean ± SD. **(D)** Immunofluorescence staining of CHO cells overexpressing GFP-hPRL-1, GFP-hPRL-2, or GFP-hPRL-3 using PRL3-zumab. *Red,* PRL3-zumab signal. *Green*, GFP-hPRL signal. *Bar*, 40 μm.




**Supplemental Figure 3.** PRL3-zumab, but not human IgG isotype control, suppresses PRL-3-positive gastric tumor growth *in vivo*. Eight-week old male BALB/C nude mice were implanted with PRL-3-positive SNU-484 cell lines to induce orthotopic gastric tumors. The dot plot indicates the mean tumor volume of SNU-484 tumors in untreated, human IgG-treated (hIgG), and PRL3-zumab-treated mice. $p < 0.0001$, one-way ANOVA; $n = 4$ per group, data representing mean ± SEM. ***$p < 0.001$, Tukey's post-hoc test (untreated vs treated groups).




**Supplemental Figure 4.** PRL3-zumab blocks orthotopic PRL-3$^+$ gastric tumors. Eight-week old male BALB/C *nude* mice were implanted with PRL-3$^+$ IM-95 or NUGC-4 gastric cancer cells to induce orthotopic PRL-3$^+$ gastric tumors. At the end of the experiment, visible tumors (outlined in black) were measured and volumes compared. **(A)** Stomachs with IM-95 tumors from untreated and PRL3-zumab-treated mice. *Bar*, 10 mm. *Rightmost panel*, mean tumor volumes. $p = 0.008$, *t*-test; $n = 6$ per group, data representing mean ± SEM. **(B)** Stomachs with NUGC-4 tumors from untreated and PRL3-zumab-treated mice. *Bar*, 10 mm. *Rightmost panel*, mean tumor volumes. $p = 0.03$, *t*-test; $n = 4$ per group, data representing mean ± SEM.




**Supplemental Figure 5.** PRL3-zumab inhibits local and metastatic abdominal tumors formed by PRL-3$^+$ HCT116 colorectal cancer cells implanted within the stomach. HCT116-luc2 cells were implanted into the gastric subserosa layer of mice stomachs to mimic secondary colorectal cancer metastasis to the gastric niche. PRL3-zumab treatment reduced growth of HCT116-luc2 tumors in the gastric niche. **(A)** IVIS imaging of global *in vivo* tumor growth over 3 weeks post-inoculation. **(B)** Mice from **(A)** were analyzed for whole-animal IVIS intensity changes over time. $n = 4$ per group; $p < 0.001$, two-way ANOVA. **(C)** Tumor burden in excised stomachs at the end of week 3. **(D)** Stomachs from **(C)** were analyzed for differences in IVIS intensity. $n = 4$ per group; $p = 0.01$, *t*-test; data representing mean ± SEM. **(E)** Metastatic tumor burden within abdominal walls at the end of week 3. **(F)** Stomachs from **(E)** were analyzed for differences in IVIS intensity. $n = 4$ per group; $p = 0.0003$, *t*-test; data representing mean ± SEM.

**Supplemental Table 1.** Clinical characteristics of SGset1 gastric cancer patient cohort.

| | **SGset1 (*n* = 185)** |
|---|---|
| Age (years) | |
| Range | 23.4 – 92.9 (1 missing) |
| Mean ± S.D. | 64 ± 12.9 (1 missing) |
| Gender (%) | |
| Male | 68 (36.8) |
| Female | 116 (62.7) |
| Missing | 1 (0.54) |
| Stage (%) | |
| 1 | 29 (15.7) |
| 2 | 30 (16.2) |
| 3 | 66 (35.7) |
| 4 | 59 (31.9) |
| Missing | 1 (0.54) |
| Lauren's histopathology (%) | |
| Intestinal | 92 (49.7) |
| Diffuse | 72 (38.9) |
| Mixed/unclassifiable | 20 (10.8) |
| Missing | 1 (0.54) |
| Helicobactor Pylori status (%) | |
| Positive | 59 (31.9) |
| Negative | 37 (20.0) |
| Missing | 89 (48.1) |
| Median overall survival (months) | 22.5 (1 missing) |
| Number of overall death events | 110 (2 missing) |

**Supplemental Table 2.** Univariate and multivariate Cox regression analysis of PRL-3 expression in SGset1 cohort.

| | **Category** | **HR (95% C.I.)** | ***p*-value** |
|---|---|---|---|
| **Univariate Cox** (PRL-3 expression) | Med vs Low | 2.35 (1.42 – 3.87) | 0.0008 |
| | High vs Low | 1.94 (1.17 – 3.21) | 0.01 |
| **Multivariate Cox** (PRL-3 expression, tumor stage) | Med vs Low | 1.99 (1.19 – 3.33) | 0.009 |
| | High vs Low | 1.76 (1.76 – 2.95) | 0.03 |

**Supplemental Table 3.** ANOVA analysis of SNU-484 tumor volume after treatment (from Main Figure 4A).

| **Factor assessed** | ***p* value**[a] |
|---|---|
| Control vs PRL3-zumab | < 0.001 |
| Control vs PRL3-zumab/5-FU | < 0.001 |
| Control vs 5-FU | < 0.001 |
| PRL3-zumab vs control | < 0.001 |
| PRL3-zumab vs PRL3-zumab/5-FU | < 0.001 |
| PRL3-zumab vs 5-FU | < 0.001 |
| PRL3-zumab/5-FU vs control | < 0.001 |
| PRL3-zumab/5-FU vs PRL3-zumab | < 0.001 |
| PRL3-zumab/5-FU vs 5-FU | < 0.001 |
| 5-FU vs control | < 0.001 |
| 5-FU vs PRL3-zumab | < 0.001 |
| 5-FU vs PRL3-zumab/5-FU | < 0.001 |

[a] *p* values calculated using post-hoc Tukey's HSD test

**Supplemental Table 4.** ANOVA analysis of WBC counts after treatment (from Main Figure 4B).

| **Factor assessed** | ***p* value**[a] |
|---|---|
| Control vs PRL3-zumab | 0.438 |
| Control vs PRL3-zumab/5-FU | < 0.001 |
| Control vs 5-FU | < 0.001 |
| PRL3-zumab vs control | 0.438 |
| PRL3-zumab vs PRL3-zumab/5-FU | < 0.001 |
| PRL3-zumab vs 5-FU | < 0.001 |
| PRL3-zumab/5-FU vs control | < 0.001 |
| PRL3-zumab/5-FU vs PRL3-zumab | < 0.001 |
| PRL3-zumab/5-FU vs 5-FU | 0.836 |
| 5-FU vs control | < 0.001 |
| 5-FU vs PRL3-zumab | < 0.001 |
| 5-FU vs PRL3-zumab/5-FU | 0.836 |

[a] *p* values calculated using post-hoc Tukey's HSD test