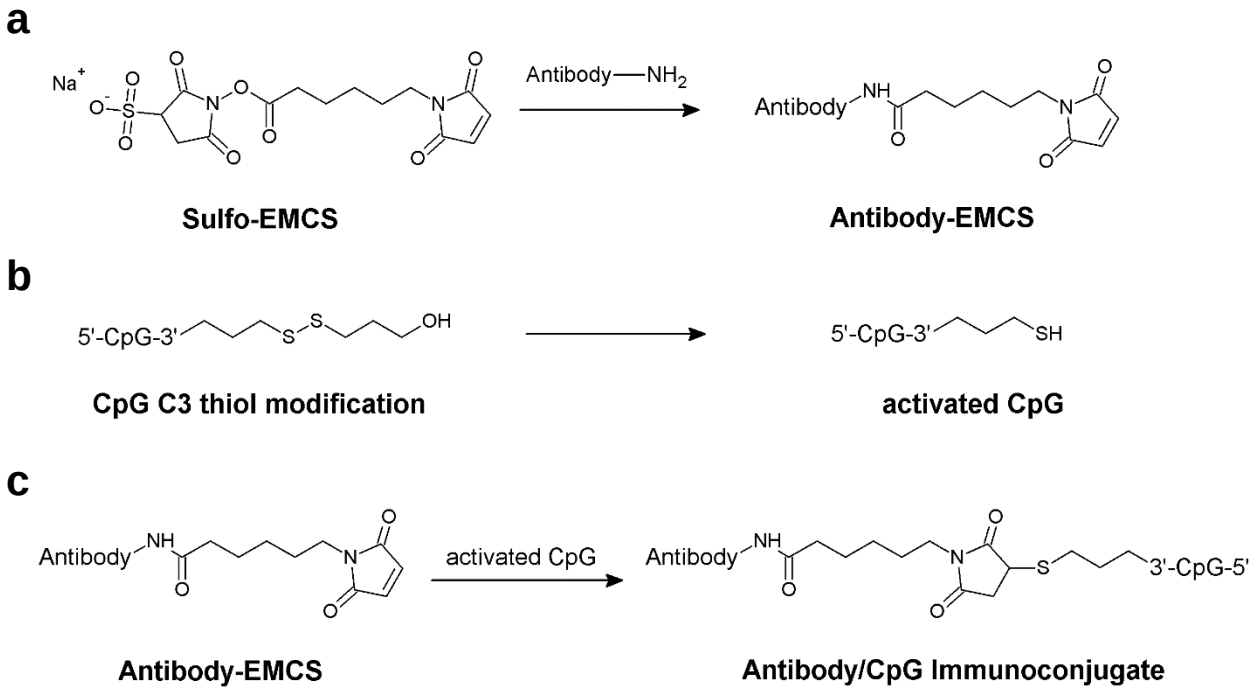
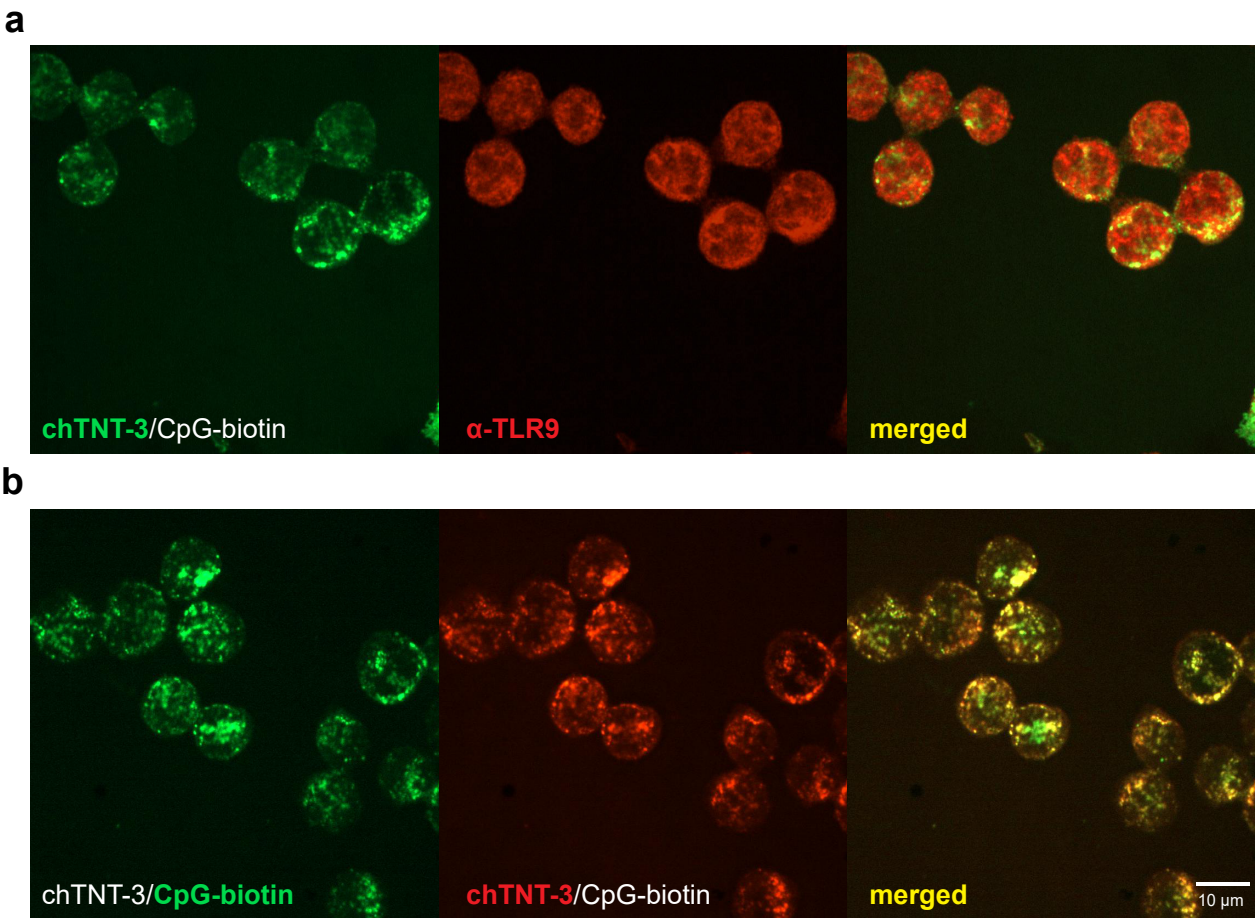
Antigen	Conjugate	Clone	Isotype	Manufacturer
CD3ε	APC-Cy7	145-2C11	Armenian Hamster IgG	Biolegend
CD4	FITC	RM4-5	Rat IgG2a	BD Biosciences
CD4	PE	GK1.5	Rat IgG2b	eBioscience
CD8α	APC	53-6.7	Rat IgG2a	eBioscience
CD25	FITC	PC61	Rat IgG1	BD Biosciences
FoxP3	APC	FJK-16s	Rat IgG2a	eBioscience
IFN-γ	PE	XMG1.2	Rat IgG1	Tonbo Biosciences

Supplementary Table S1 Antibodies used in flow cytometry experiments.



Supplementary Figure S1 Immunoconjugation reactions for the generation of antibody/CpG. (a) Primary amines on the antibody reacted with the NHS ester on Sulfo-EMCS. **(b)** 3'-modified CpG were reduced to yield a free sulfhydryl group. **(c)** The sulfhydryl group on CpG reacted with the maleimide group in the antibody-EMCS conjugates to form antibody/CpG.

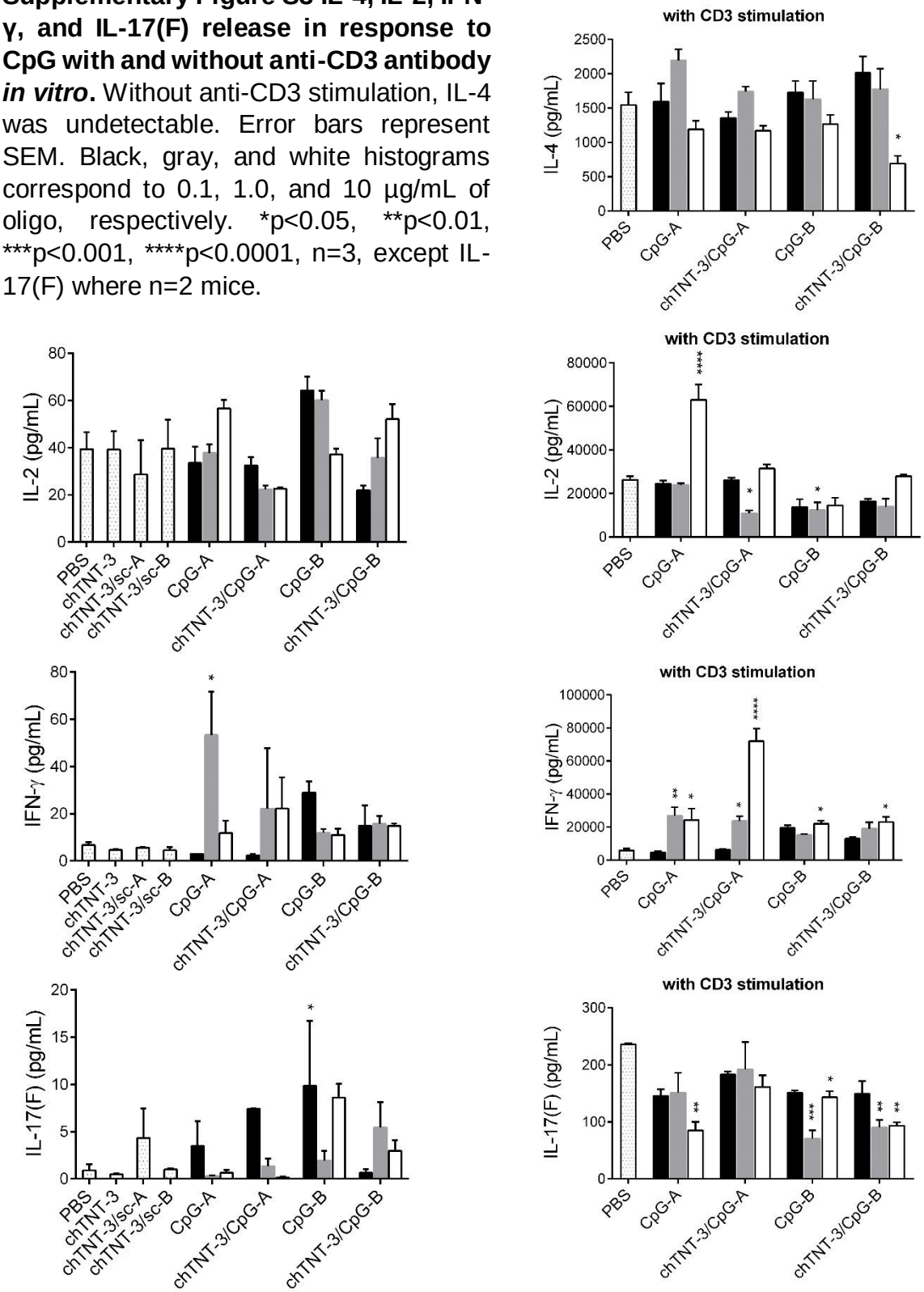
Supplementary Figure S1. Jang et al.



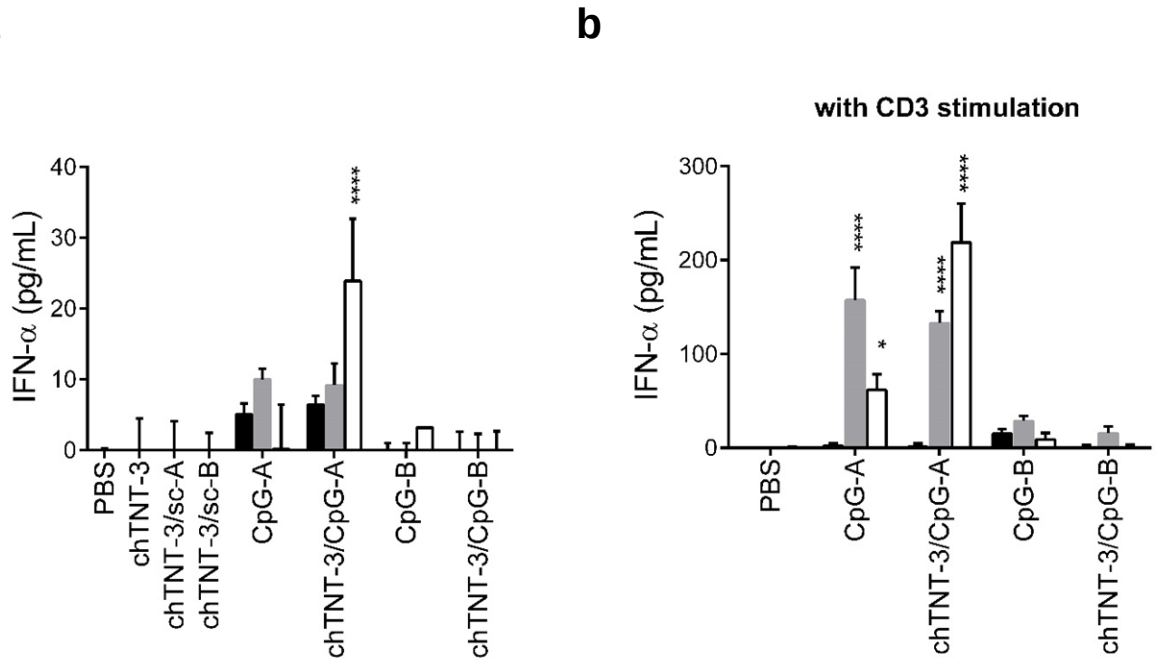
Supplementary Figure S2 Internalization and localization of chTNT-3 moiety of chTNT-3/CpG-biotin. (a) Fluorescent microscopy demonstrating internalization of chTNT-3-moiety and co-localization with TLR9 at 60 min. chTNT-3 was detected using α-huIgG-AF488 (green). TLR9 was detected using α-TLR9 and α-rabbit IgG-AF568 (red). **(b)** chTNT-3 moiety stays conjugated with the CpG-biotin moiety following internalization at 60 min. CpG-biotin was detected using streptavidin-AF488 (green) and chTNT-3 was detected using α-huIgG-AF568 (red).

Supplementary Figure S2. Jang et al.

Supplementary Figure S3 IL-4, IL-2, IFN-γ, and IL-17(F) release in response to CpG with and without anti-CD3 antibody *in vitro*. Without anti-CD3 stimulation, IL-4 was undetectable. Error bars represent SEM. Black, gray, and white histograms correspond to 0.1, 1.0, and 10 μg/mL of oligo, respectively. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, n=3, except IL-17(F) where n=2 mice.

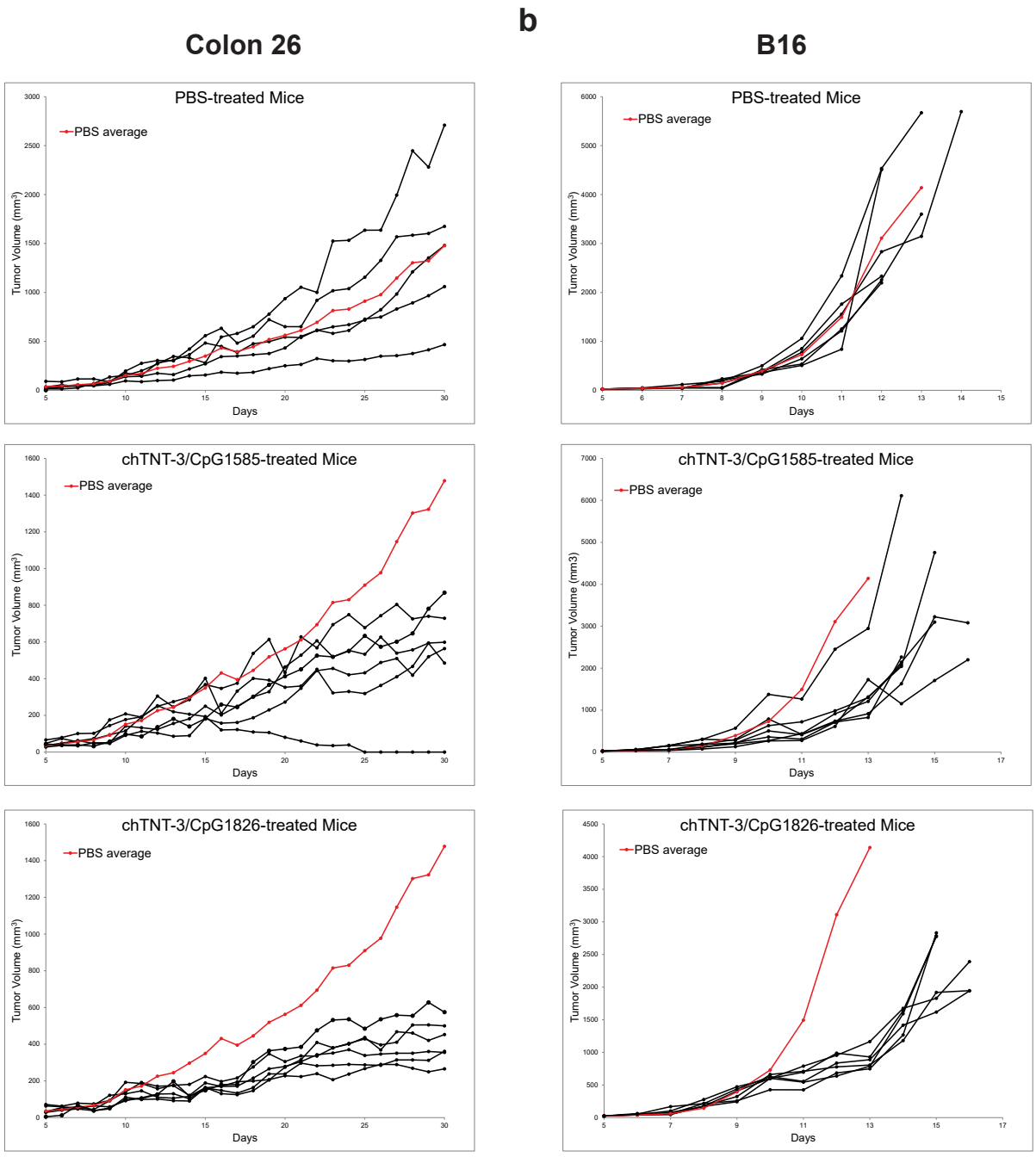


Supplementary Figure S3. Jang et al.



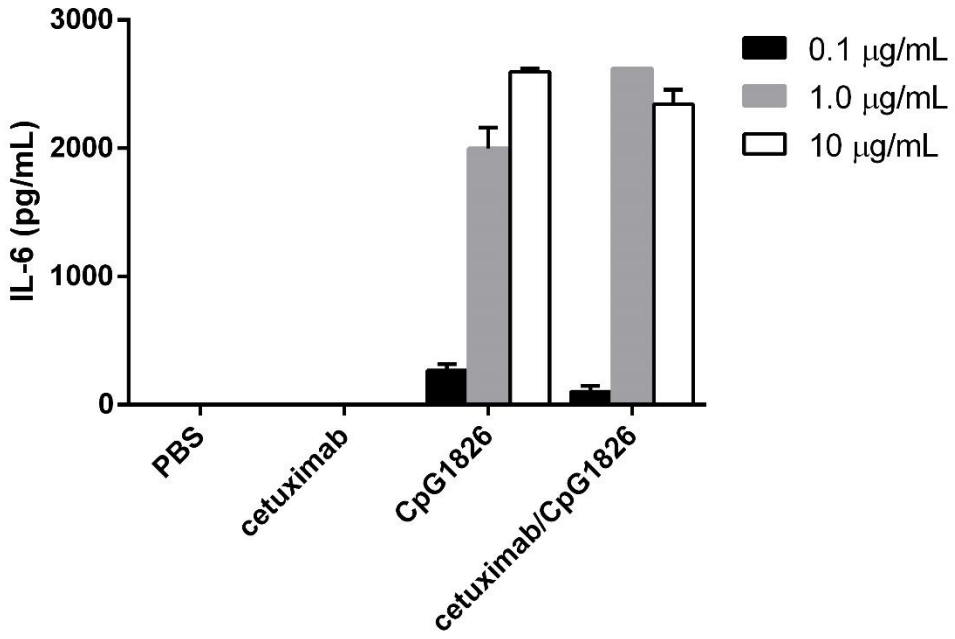
Supplementary Figure S4 Class A CpG and its immunoconjugates induced IFN-α *in vitro.* (a) Without and **(b)** with CD3 stimulation. Supernatants were analyzed by ELISA. *p<0.05, ****p<0.0001, n=3 mice.

Supplementary Figure S4. Jang et al.



Supplementary Figure S5 Individual tumor curves for mice treated with PBS, chTNT-3/CpG1585, and chTNT-3/CpG1826. (a) Colon 26 tumor model in BALB/c mice. **(b)** B16 tumor model in C57BL/6 mice.

Supplementary Figure S5. Jang et al.



Supplementary Figure S6 *In vitro* activity of cetuximab/CpG1826 on murine splenocytes. Splenocytes from a naïve BALB/c female mouse were stimulated with cetuximab, CpG1826, or cetuximab/CpG1826 in low serum medium (2.5% FBS in RPMI) for four days. Concentrations of cetuximab were 0.54, 5.4, and 54 μg/mL, corresponding to 0.1, 1.0, and 10 μg/mL of CpG. IL-6 concentrations were measured in the supernatants using Mouse IL-6 Quantikine ELISA Kit (R&D, Minneapolis, MN). Error bars represent standard error of the mean of triplicate samples.

Supplementary Figure S6. Jang et al.