

Supporting Information

Optimized protocol for quantitative multiple reaction monitoring-based proteomic analysis of formalin-fixed, paraffin embedded tissues

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Contents

Supplemental Methods.

Figure S1: Preparation of samples was designed to minimize the effects of tissue heterogeneity.

Figure S2: Response curves for assays measured in FFPE and frozen tissue. *Note: due to size, Supplemental Figure S2 is appended at the end of this document.*

Figure S3: Preparation of breast cancer tumor tissue samples for comparison of protein quantification in fresh frozen and FFPE samples was designed to minimize the effects of tissue heterogeneity.

Figure S4: Comparison of endogenous peptide measurements for lysine-containing and arginine-containing peptides in frozen and FFPE samples.

Table S1: Analytical performance figures of merit for LC-MRM and immuno-MRM assays characterized in frozen and FFPE samples.

SUPPLEMENTAL METHODS

Digestion sample desalt. Digests were desalted using an Oasis HLB 96-well Plate with 5 mg sorbent per well (Waters Cat. #186000309). The wells were washed with 3 volumes of 80% MeCN/0.1% formic acid (FA), and then equilibrated with 4 washes of 0.1% FA. The digest was applied to a well, then washed with 4 volumes 0.1% FA before being eluted with 3 washes of 80% MeCN/0.1%FA. The eluate was then aliquotted by volume and digests were lyophilized, followed by storage at -80°C until use.

Antibody cross-linking to Protein G magnetic beads. The antibodies were covalently coupled to 37-100 µm Protein G magnetic sepharose beads (GE Healthcare Life Sciences, Cat.# 28-9513-79) as follows. Each antibody was coupled and immobilized separately using an antibody-bead ratio of 5 µg antibody to 1 µL beads. Eighty microliters of beads were added to microcentrifuge tubes, the supernatants were discarded, and 1X PBS, 5% CHAPS (final concentration 0.03%), and 400 µg of mAb were added to arrive at a final volume of 900 µL, and the tubes were tumbled overnight at 4 °C. (Larger volumes and tubes were used if the concentrations of the purified mAbs were low; after overnight coupling, excess supernatant was removed to arrive at 900 µL.) Automated immobilization was subsequently performed using a KingFisher magnetic bead handling platform (5400500, Thermo Scientific, Waltham, MA) equipped with a deep well magnet head and using 96 deep well plates (95040450, Thermo Scientific) and 96 tip combs for deep well magnets (97002534, Thermo Scientific). The KingFisher was moved into a fume hood for the immobilization procedure due to the toxicity of DMP (dimethyl pimelimidate dihydrochloride, Sigma, Cat.# D8388), triethanolamine (Sigma, Cat.# T1377), and monoethanolamine (Sigma, Cat.# 411000). Seven plates were used, with the following contents per plate and well, and using the following mixing times: plate 1, 900 µL of the antibody-coupled beads that had been transferred from the microcentrifuge tubes into the plate (5 min); plate 2, 900 µL of freshly-made 20 mM DMP in 200 mM triethanolamine, pH 8.5 (30 min); plate 3, 900 µL of 150 mM monoethanolamine, pH 9.0 (30 min); plates 4 and 5, 900 µL of 1X PBS-0.03% CHAPS (5 min); plate 6, 800 µL of 1X PBS-0.03% CHAPS-0.1% sodium azide (5 min); plate 8, tip comb. A medium mixing speed was used for each plate. The antibody-beads were left in the 800 µL of 1X PBS-0.03% CHAPS-0.1% sodium azide in plate 6 at the end of the method (at a final antibody concentration of 0.5 µg/µL). The individual antibody-bead suspensions were subsequently transferred to separate, new 1.5 mL screw-cap tubes (72.692, Sarstedt, Nümbrecht, Germany) and kept at 4 °C until use. For ESR1 (peptide LLFAPNLLDR), 2000 µg of antibody was coupled to 400 µL of magnetic beads (more antibody was coupled for it than for the other antibodies, since 10 µg of it was used per capture (see below), versus 1 µg of the other antibodies).

Peptide immunoaffinity enrichment. Peptide immunoaffinity enrichment for all experiments was performed on at least 25 µg of digested cell lysate and consisted of overnight incubations of the cross-linked monoclonal antibodies (mAbs) using a mix of the 42 mAb prepared with a concentration of 1 µg of each mAb per 50 µL 1X PBS-0.03% CHAPS-0.1% sodium azide (except for the ESR1 (LLFAPNLLLDR) antibody, for which 10 µg per 50 µL was used). The lyophilized protein digests were resuspended in 200 µL of 1X PBS-0.01% CHAPS (pH 7), and the pH was tested to ensure a pH ~7. Subsequently, 50 µL of the 42 mAb mix was added to each sample. The captures were performed in 96-well plates (97002540, Fisher Scientific) that were covered with plastic mats (Thermo Scientific, Cat.# 4421) and then tumbled overnight at 4°C. A KingFisher platform was used to wash the beads as described previously¹ with the following modifications: the beads were washed twice with 250 µL of 1X PBS-0.01% CHAPS, pH 7, and once with 250 µL of 1/10X PBS-0.01% CHAPS, pH 7), and the peptides were eluted using 26 µL of 3% acetonitrile-5% acetic acid-50 mM citric acid. A PCR plate (HSP9601, BioRad, Hercules, CA) was used for the peptide elution step. Ten microliters of an eluate were injected for an LC-MRM-MS analysis (immuno-MRM).

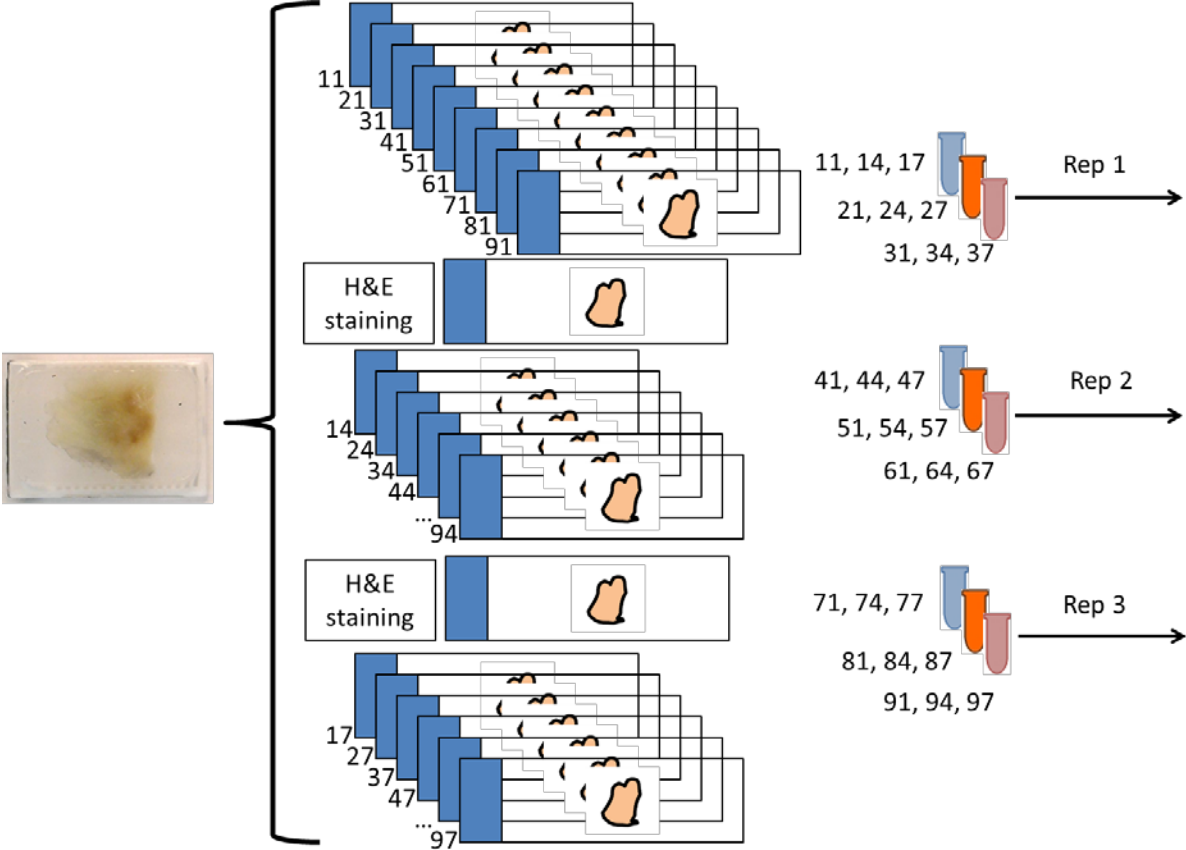
MRM parameter optimization. Heavy stable isotope-labeled standards (SIS) and matched light versions were synthesized and purified to >95% purity by HPLC. Heavy peptides incorporated a fully atom labeled ¹³C and ¹⁵N isotope at the C-terminal lysine (K) or arginine (R) position of each (tryptic) peptide, resulting in a mass shift of +8 or +10 Da, respectively. Peptides were quantified by amino acid analysis and aliquots were stored in 5-30% acetonitrile/0.1% formic acid at -80°C until use. Transitions monitored by LC-MRM were selected and optimized as previously reported.² Briefly, each synthetic peptide was analyzed by MS/MS on an LTQ mass spectrometer using infusion with an Advion TriVersa interface. The spectral library from these analyses was used to select transitions for optimization. Optimal collision energy for a hybrid triple quadrupole/linear ion trap mass spectrometer (5500 QTRAP) for each of the peptides was determined by injecting 50 fmol standard peptide solutions into a flow of 30% acetonitrile, 0.1% formic acid at a flow rate of 1 µL/min. Optimal values were determined by ramping the potentials and evaluating the results through DiscoveryQuant (Sciex). These optimized values were used to prepare linear regressions provided in Skyline for collision energy (CE) depending on the molecular weight and charge state of the peptides by using slope and intercept parameters of 0.0528 and -2.1786 for charge state +2, and 0.0448 and -1.2844 for charge state +3. Transitions monitored by immuno-MRM were optimized by using these linear regression values for the CE and were selected by injecting 50 fmol of a synthetic peptide mix (in 3% acetonitrile-0.1% formic acid) onto the LC-MRM-MS system. A static declustering potential (DP) of 100 V was used for all transitions.

Retention times on the LC platform were empirically determined using a mixture of the standard stable isotope-labeled synthetic peptides in a non-scheduled fashion. Once the retention times were known, scheduled MRMs were set up using a 150 second MRM detection window and a target cycle time of 1.5 seconds.

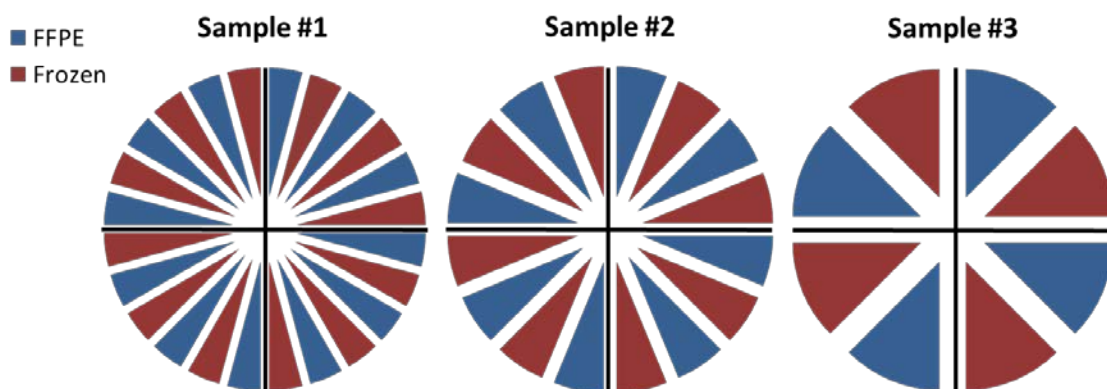
MRM mass spectrometry data analysis. MRM peak integration was performed by Skyline⁴, and the integrations were manually inspected to ensure correct peak detection, absence of interferences, and accurate integration. Reported peak areas are the sum of the peak area and background area reported by Skyline. Peak specificity between the light (or endogenous) and heavy (or SIS) MRM signal was defined as the detection of ≥ 1 transition from the endogenous peptide exactly co-eluting with ≥ 2 transitions from the stable isotope-labeled peptide, with the relative intensity of the light transition(s) deviating no more than 20% compared to the relative intensity of the corresponding heavy transitions. Integration results were exported to the program R for linear regression and statistical analysis. Peptide concentrations are calculated as the peak area ratio of the sum of all transitions with no interferences times the concentration of SIS peptide.

- (1) Schoenherr, R. M.; Whiteaker, J. R.; Zhao, L.; Ivey, R. G.; Trute, M.; Kennedy, J.; Voytovich, U. J.; Yan, P.; Lin, C.; Paulovich, A. G. Multiplexed quantification of estrogen receptor and HER2/Neu in tissue and cell lysates by peptide immunoaffinity enrichment mass spectrometry. *Proteomics* **2012**, *12* (8), 1253–1260.
- (2) Kennedy, J. J.; Abbatiello, S. E.; Kim, K.; Yan, P.; Whiteaker, J. R.; Lin, C.; Kim, J. S.; Zhang, Y.; Wang, X.; Ivey, R. G.; et al. Demonstrating the feasibility of large-scale development of standardized assays to quantify human proteins. *Nat. Methods* **2014**, *11* (2), 149–155.
- (3) Maclean, B.; Eng, J. K.; Beavis, R. C.; McIntosh, M. General framework for developing and evaluating database scoring algorithms using the TANDEM search engine. *Bioinformatics* **2006**.
- (4) MacLean, B.; Tomazela, D. M.; Shulman, N.; Chambers, M.; Finney, G. L.; Frewen, B.; Kern, R.; Tabb, D. L.; Liebler, D. C.; MacCoss, M. J. Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinforma. Oxf. Engl.* **2010**, *26* (7), 966–968.
- (5) Maclean, B.; Tomazela, D. M.; Abbatiello, S. E.; Zhang, S.; Whiteaker, J. R.; Paulovich, A. G.; Carr, S. A.; MacCoss, M. J. Effect of collision energy optimization on the measurement of peptides by selected reaction monitoring (SRM) mass spectrometry. *Anal. Chem.* **2010**, *82* (24), 10116–10124.

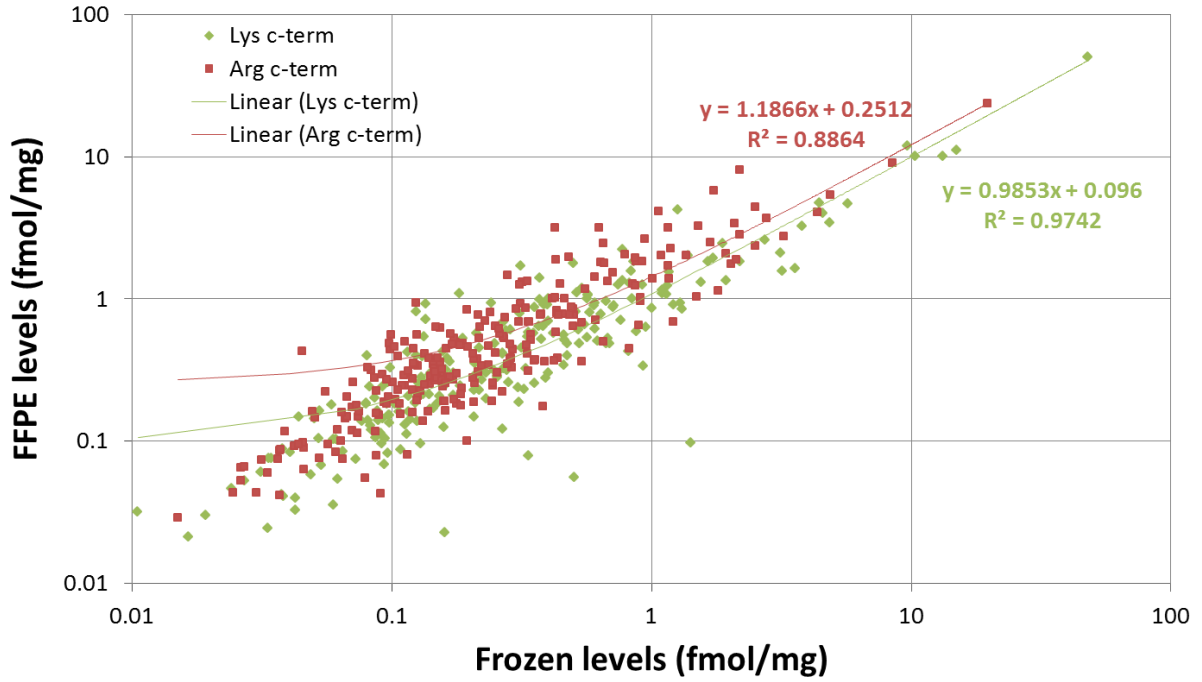
Supplemental Figure S1. Preparation of samples to minimize the effects of tissue heterogeneity. Tissue blocks embedded in paraffin were sectioned by mounting 10um slices onto slides. Alternating slides were distributed across replicates to minimize, to the largest extent possible, variations in cell type composition throughout the tissue block. In this manner, each replicate analyzed in the optimization experiment contained roughly equal tissue composition.



Supplemental Figure S3. Preparation of breast cancer tumor tissue samples for comparison of protein quantification in fresh frozen and FFPE samples was designed to minimize the effects of tissue heterogeneity. Three individual breast cancer tumor tissue samples were collected and immediately quartered. Each quarter was sectioned into pieces, with the number of pieces approximated by tumor size. Each section weighed at least 5 mg (wet weight). Alternating pieces were either flash frozen in liquid nitrogen or placed in formalin for fixation. For protein analysis, pieces were combined into one sample, and protein extraction and digestion performed in triplicate.

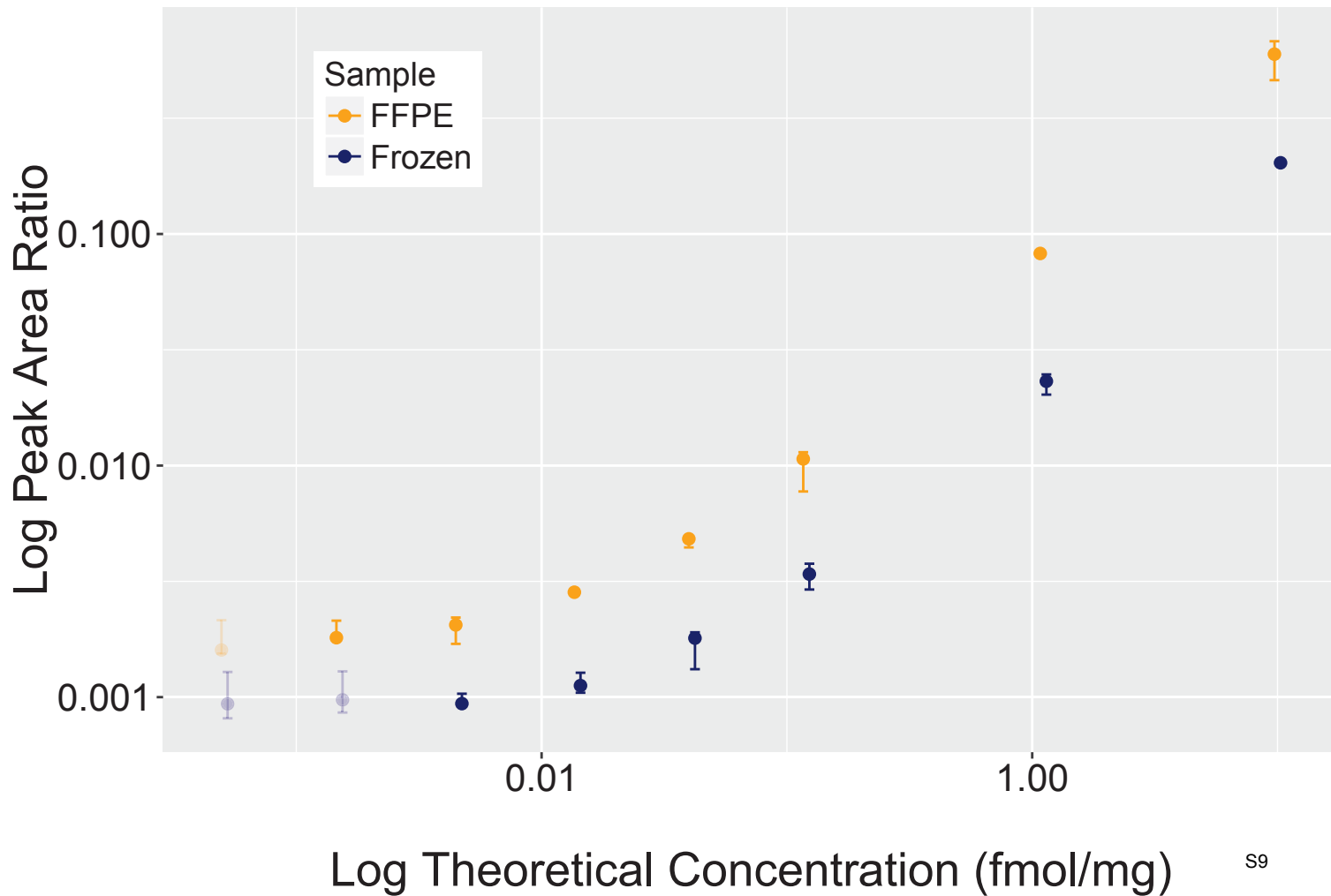


Supplemental Figure S4. Comparison of endogenous peptide measurements for lysine- and arginine-containing peptides in fresh and FFPE samples. LC-MRM quantitative results for peptides ending in lysine (K) were plotted separately from peptides ending in arginine (R). The scatter plot of peptide measurements in FFPE and frozen samples shows good correlation for both peptide types.

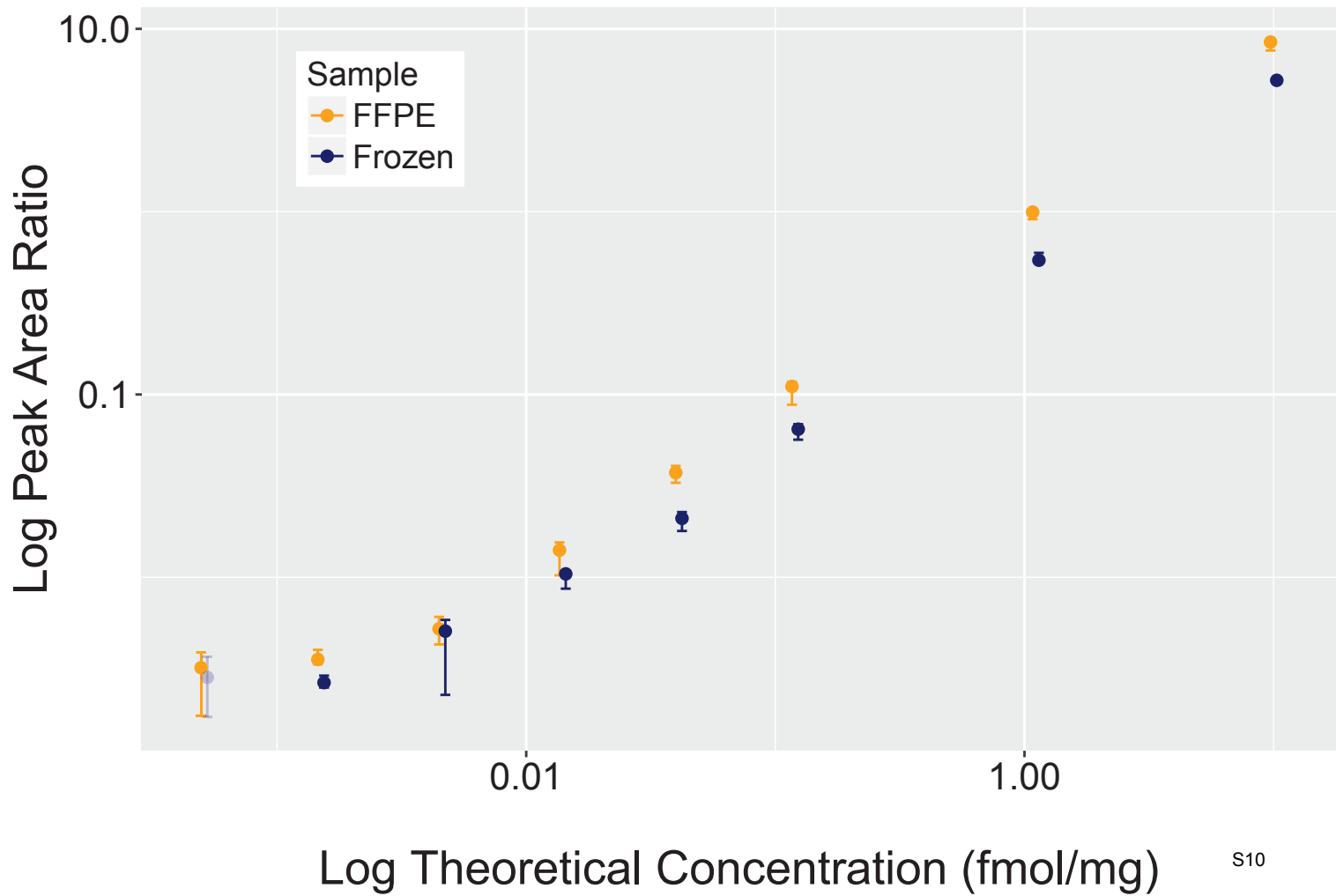


Supplemental Figure S2. Response curves for assays measured in FFPE and frozen tissue. For the following pages, peak area ratio (heavy:light) is plotted as a function of spiked heavy peptide concentration (fmol/mg) for individual peptides quantified by LC-MRM and immuno-MRM. Each page corresponds to an individual peptide. The peptide sequence and gene symbol are reported at the top of the page. The curve plots are transformed to \log_{10} scale. Values measured in FFPE background matrix are in yellow, values measured in fresh frozen tissue are in blue. Error bars are the standard deviation of triplicate measurements.

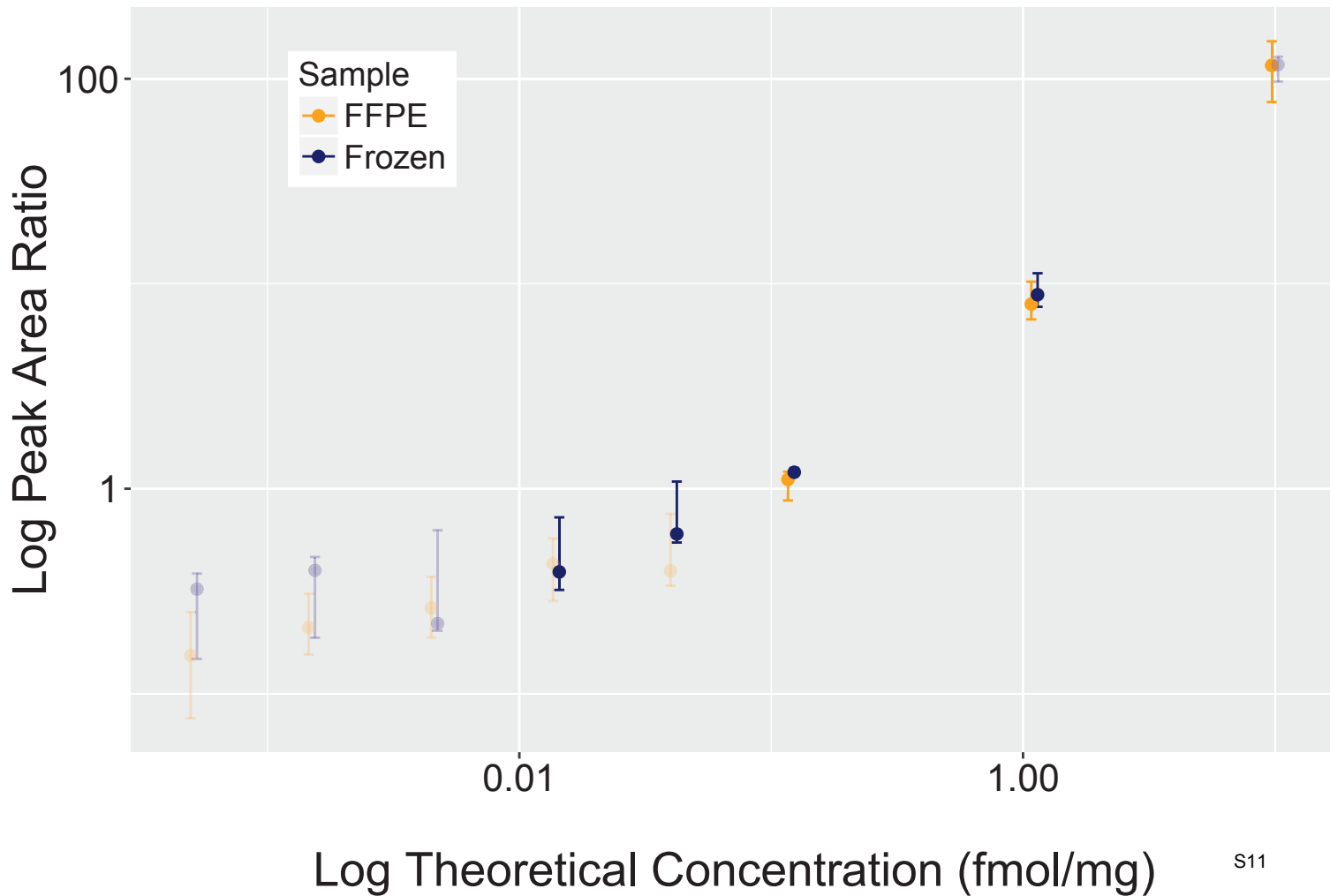
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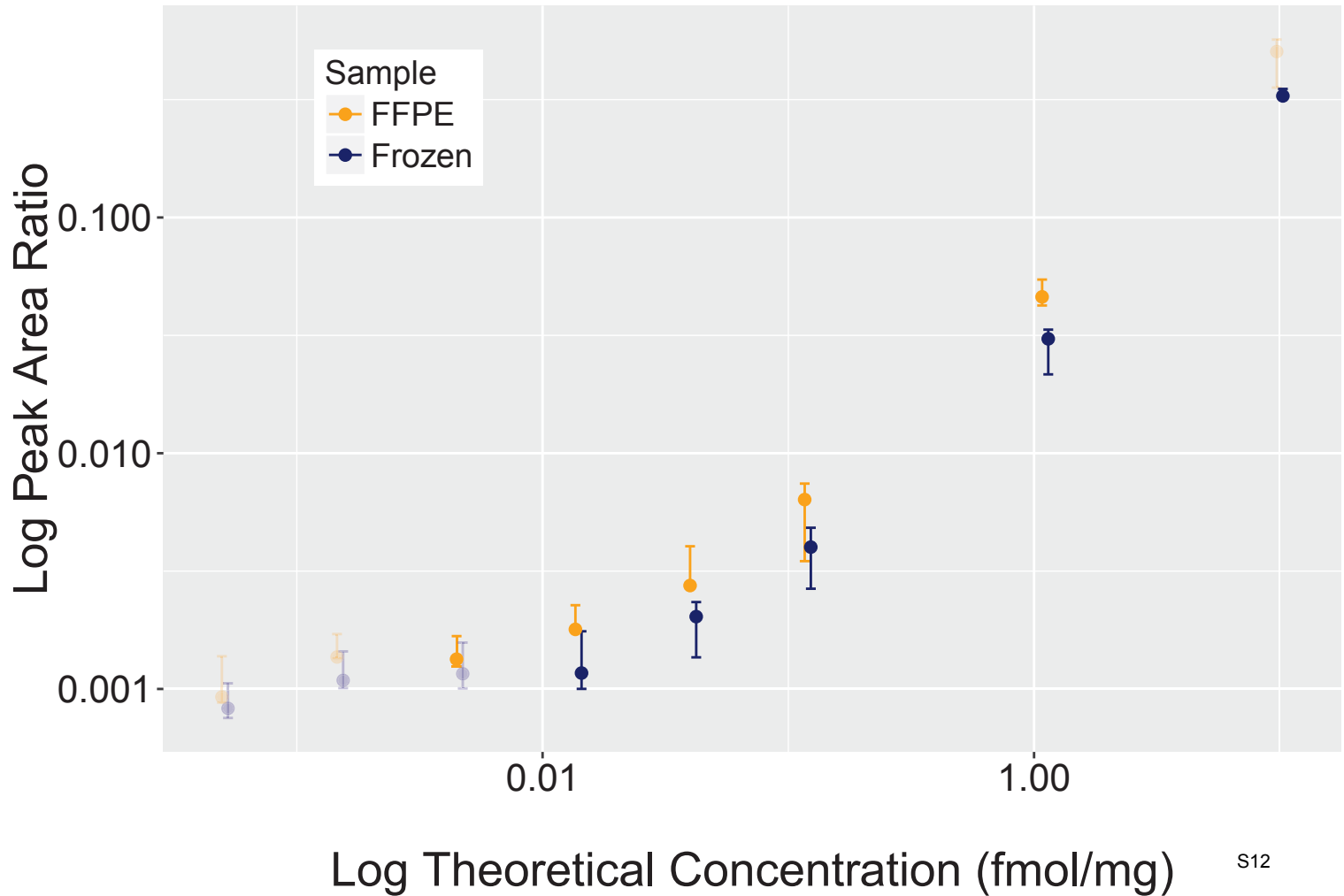
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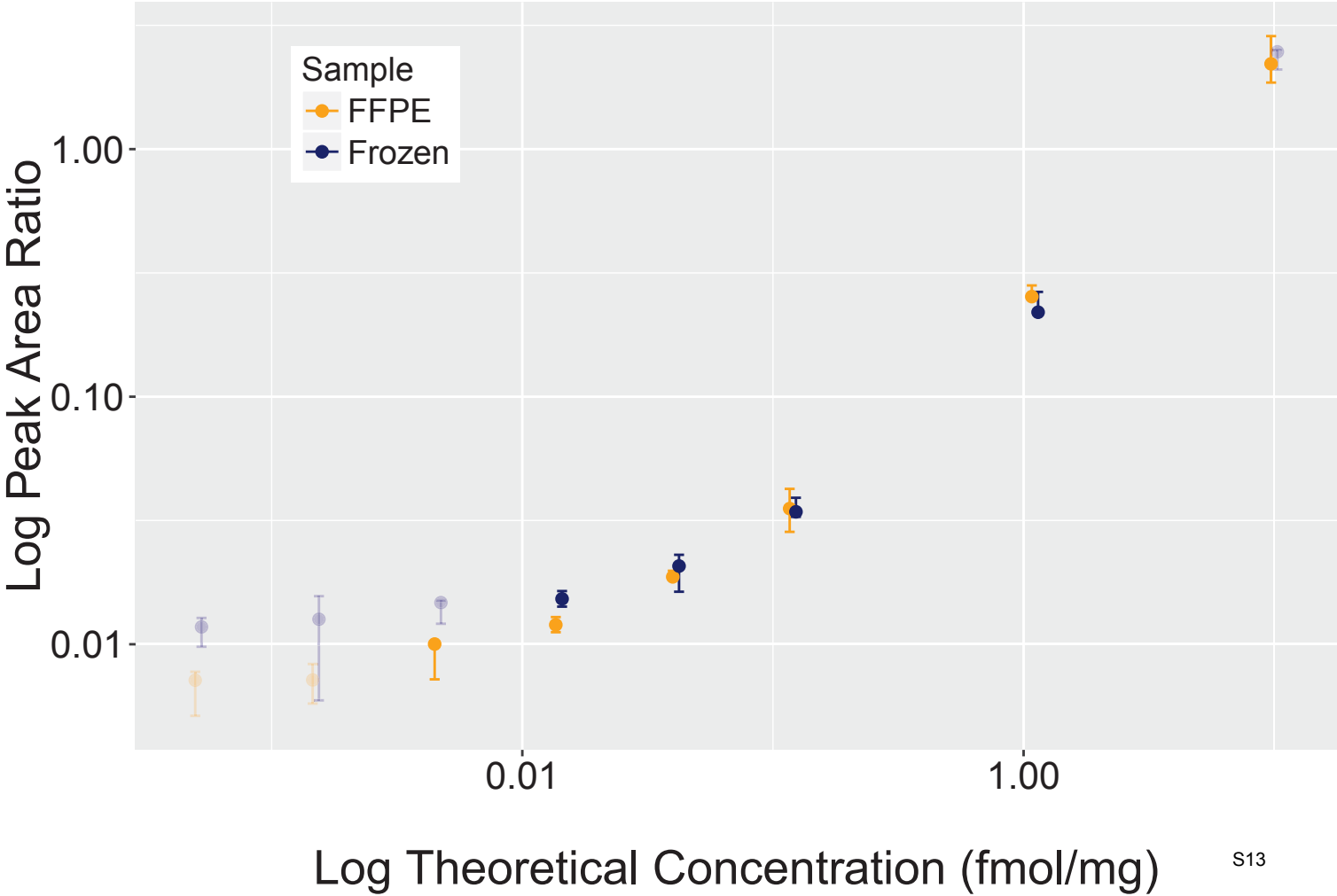
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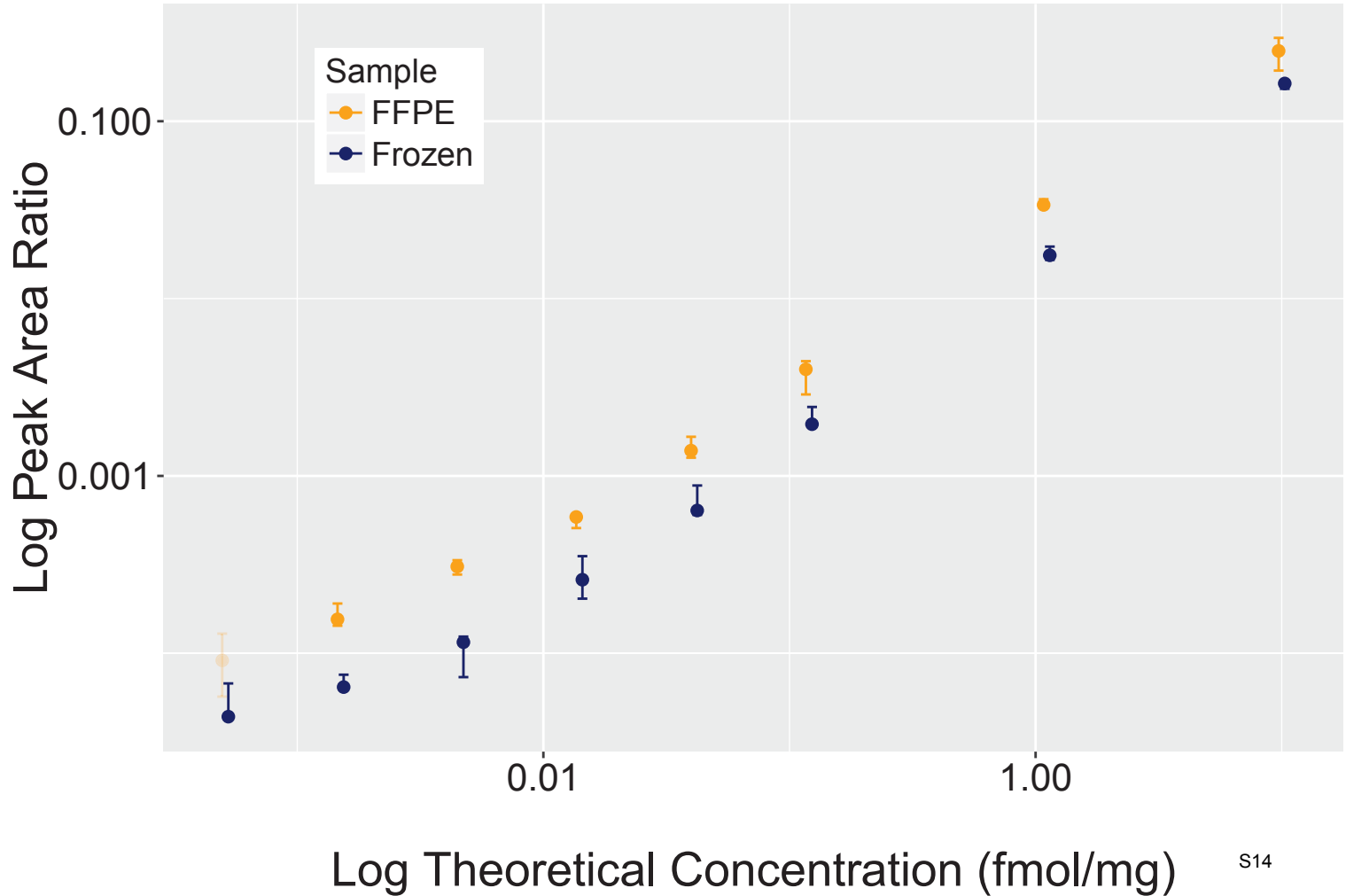
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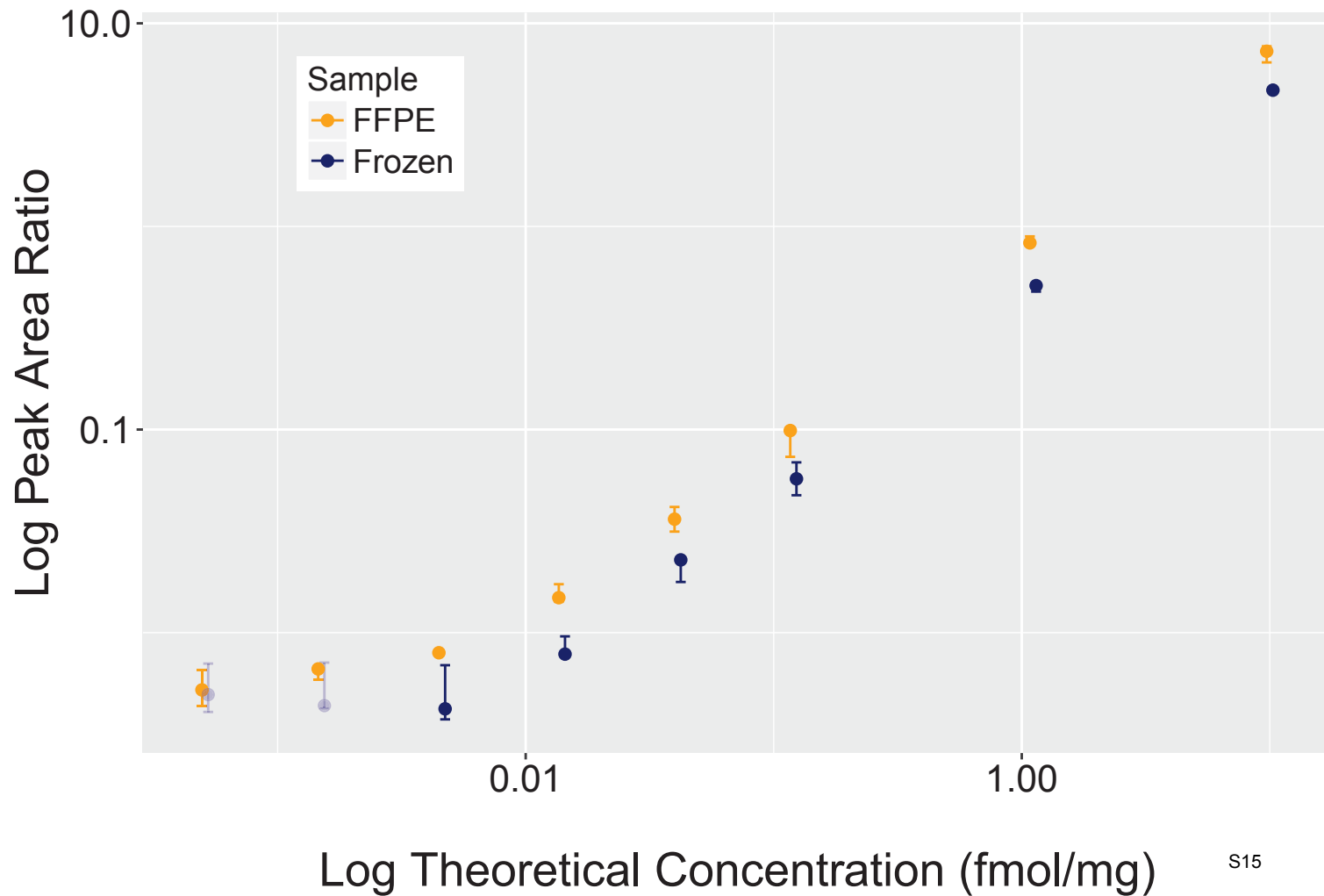
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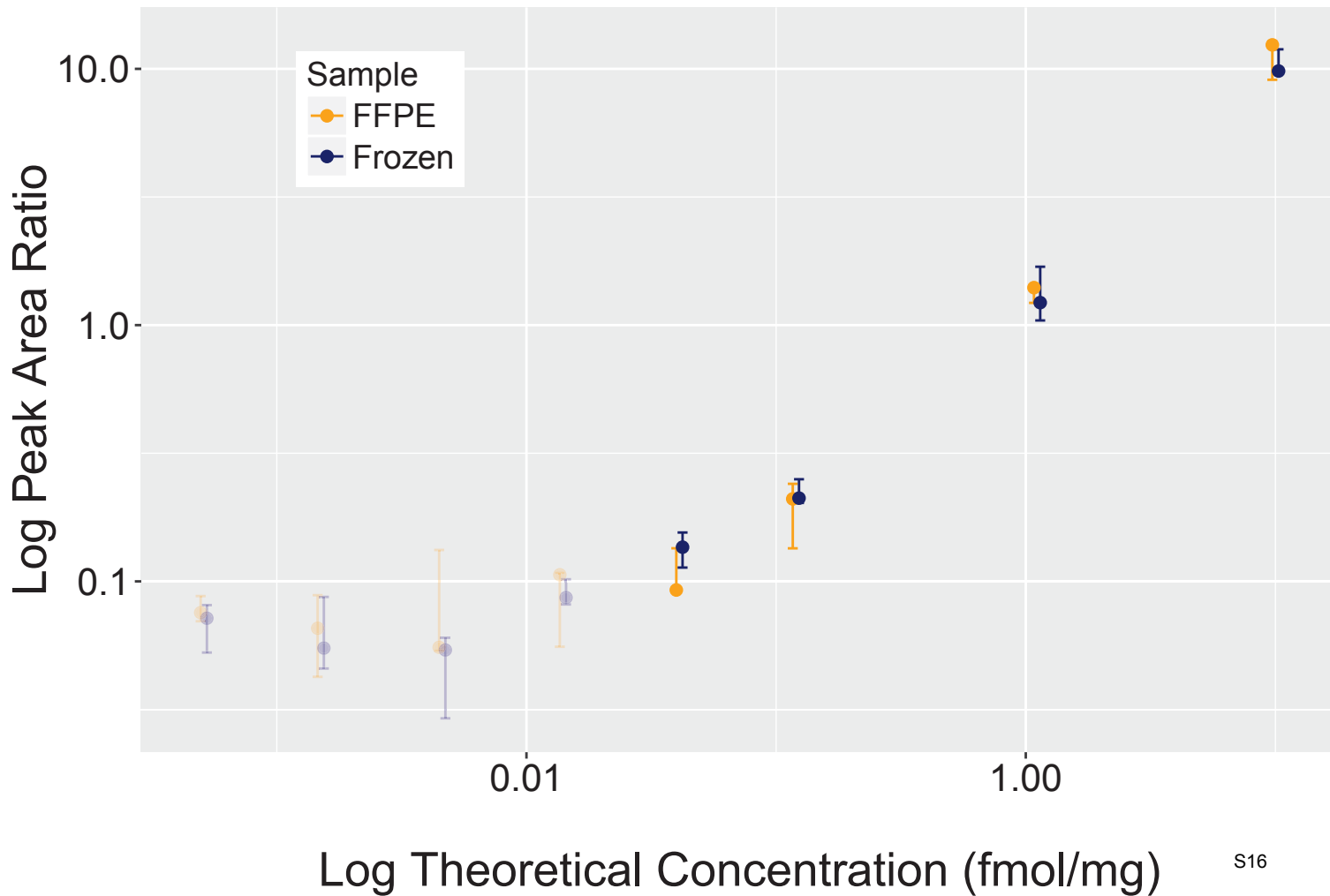
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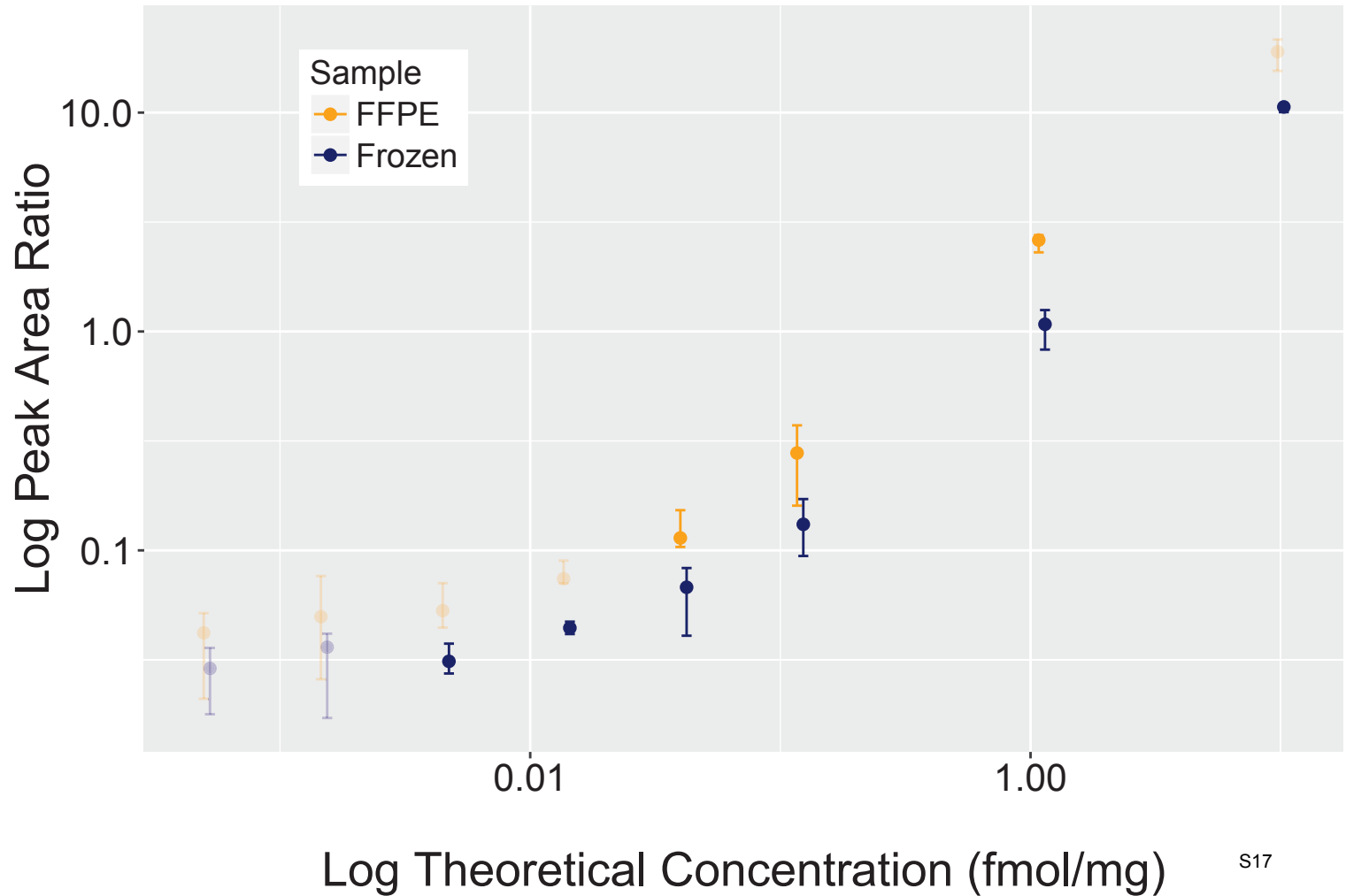
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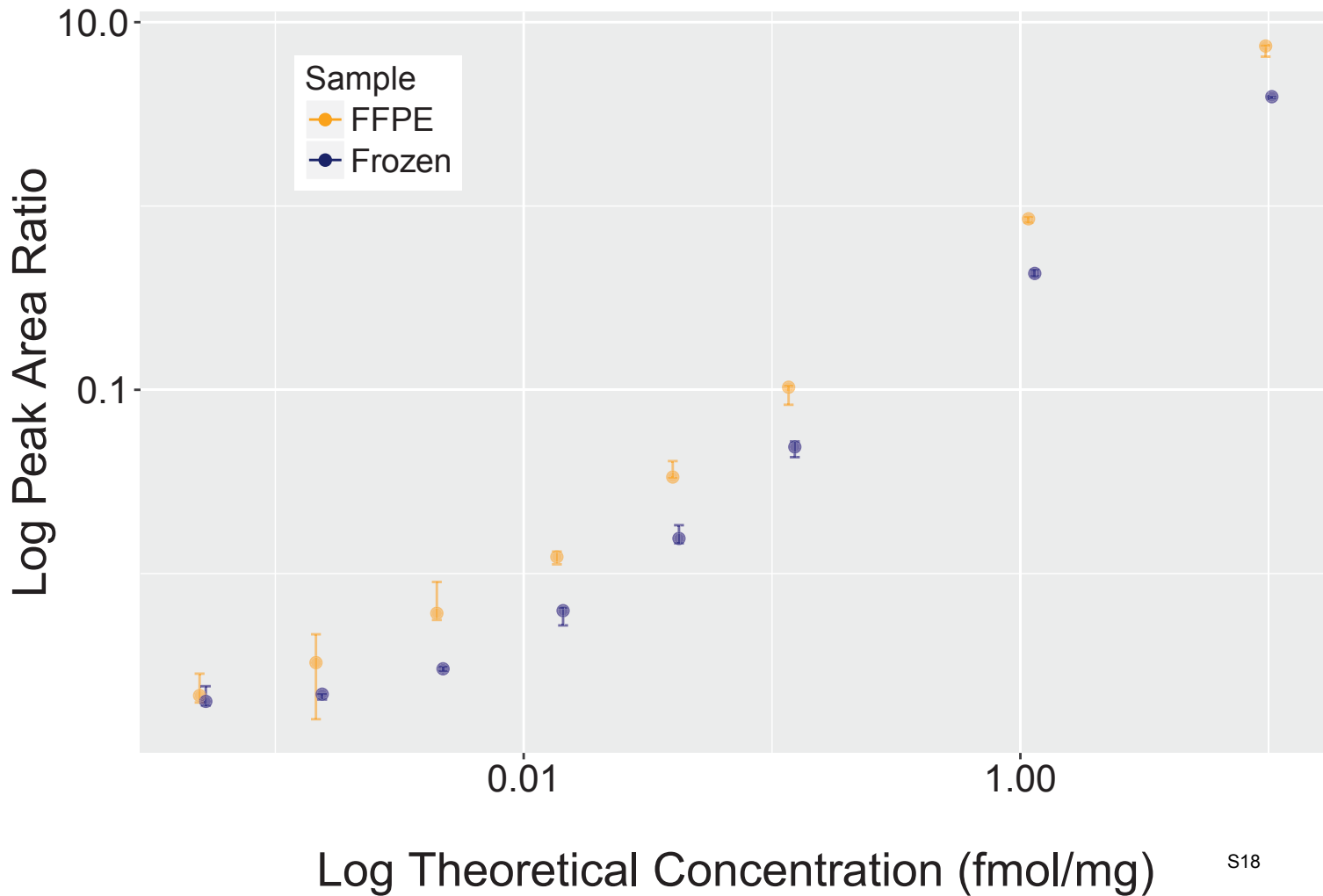
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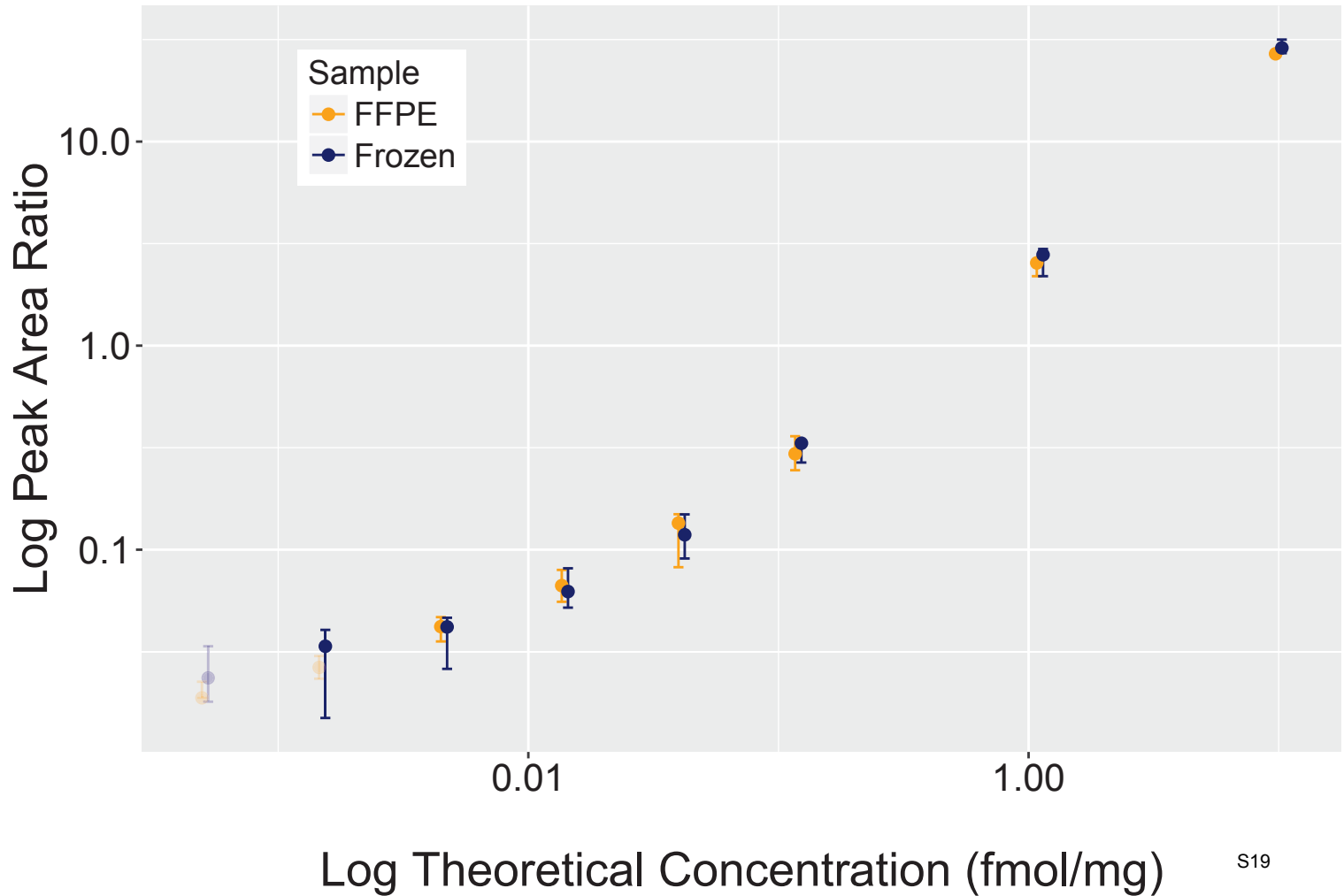
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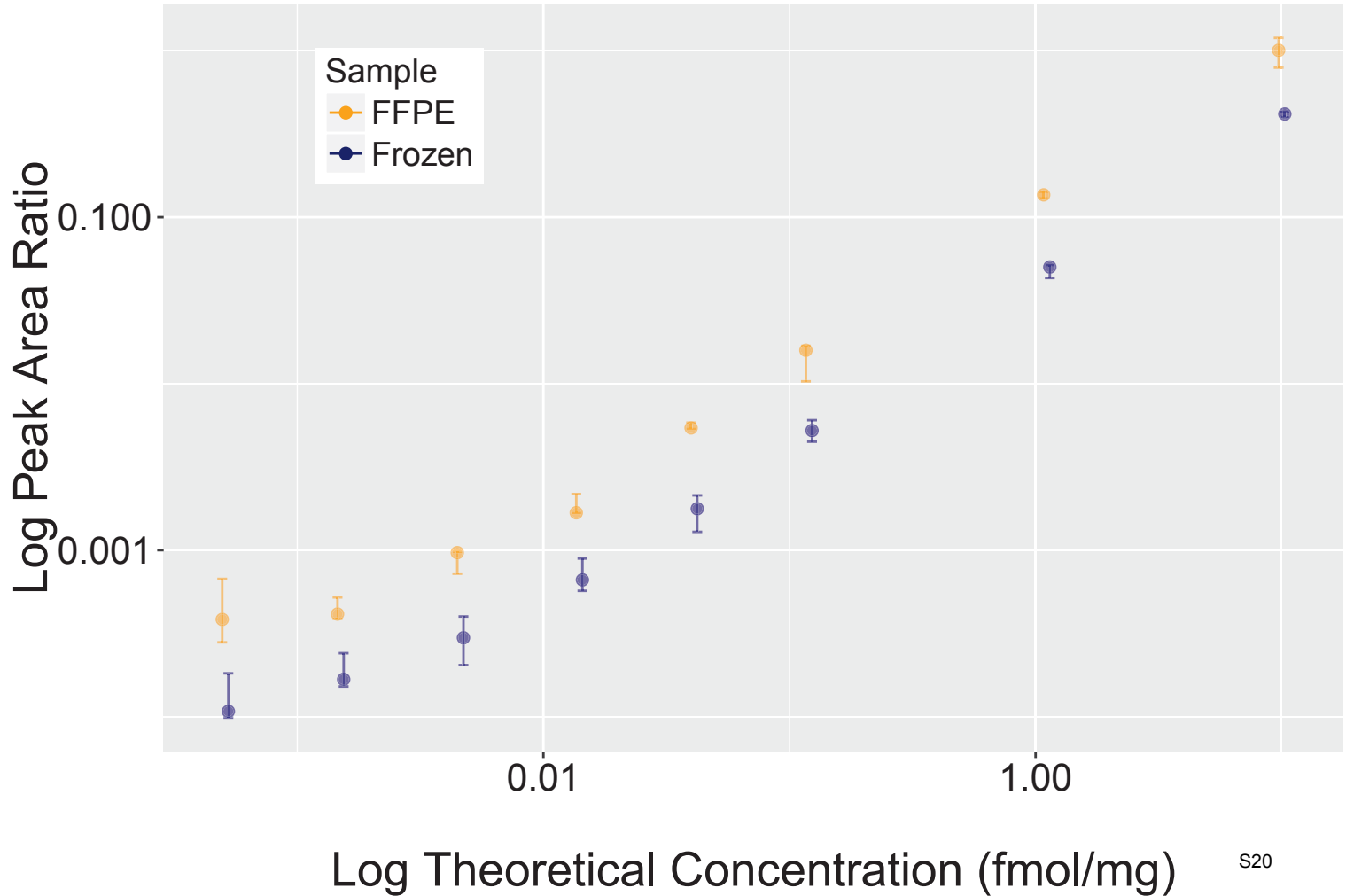
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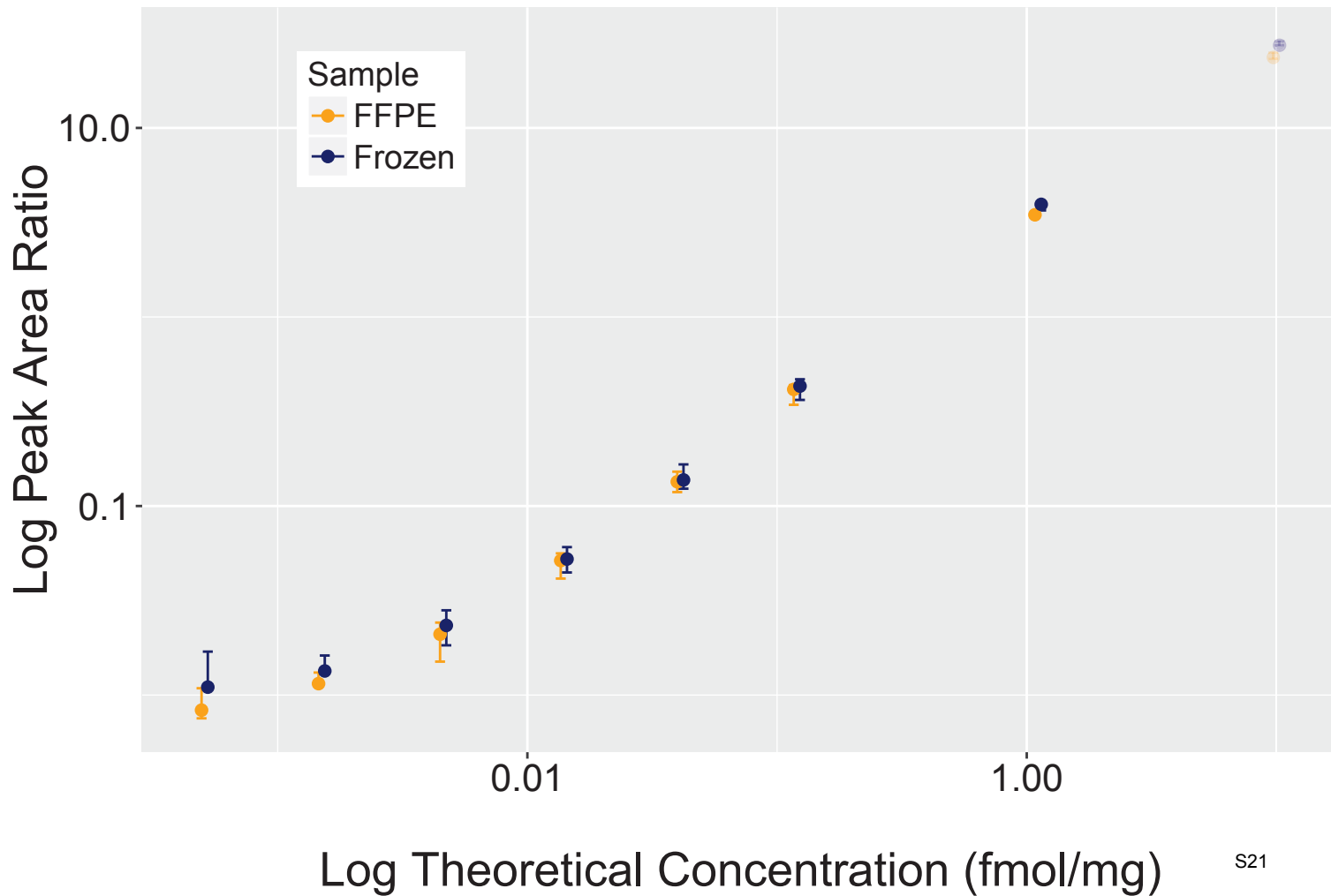
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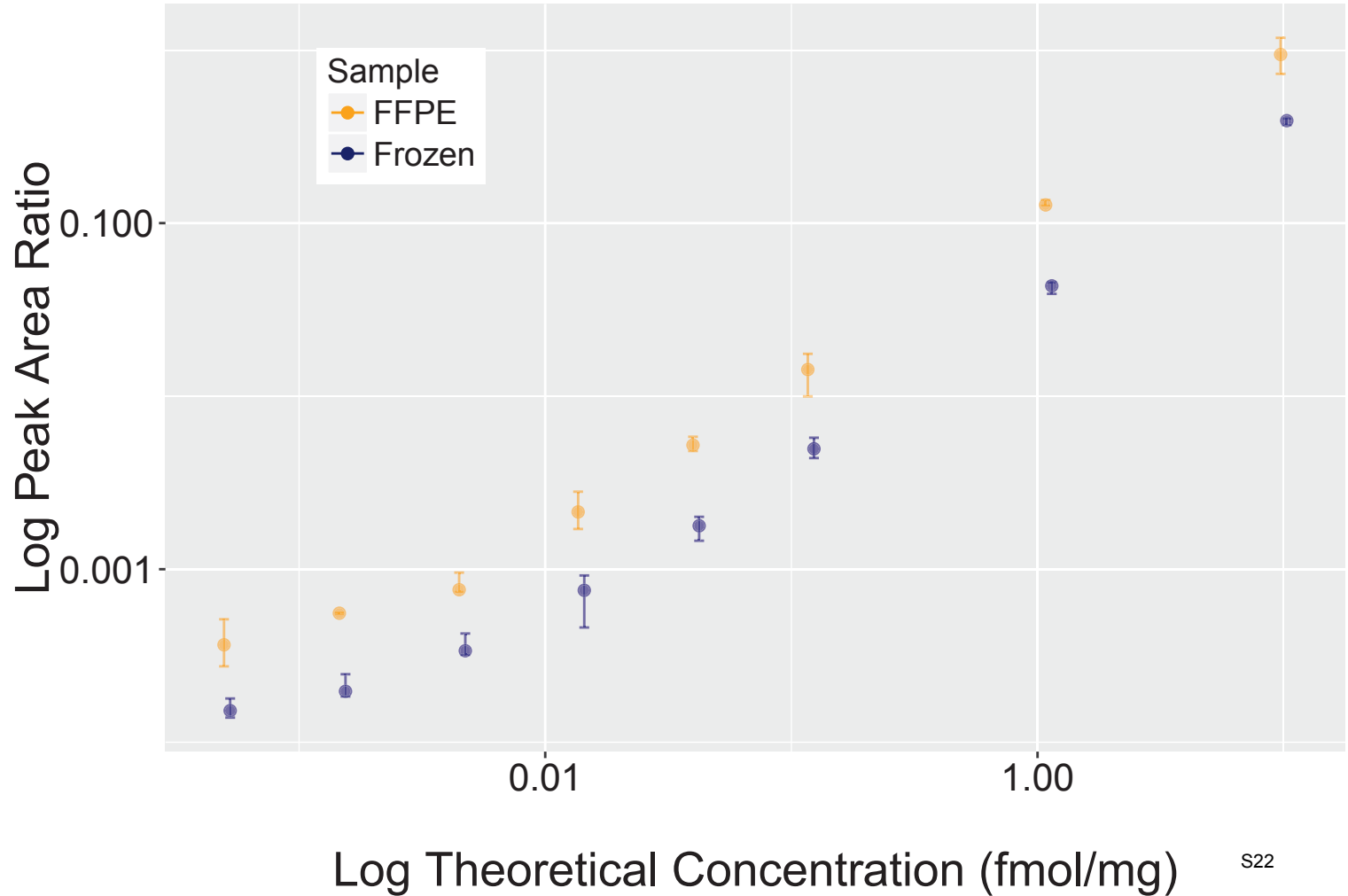
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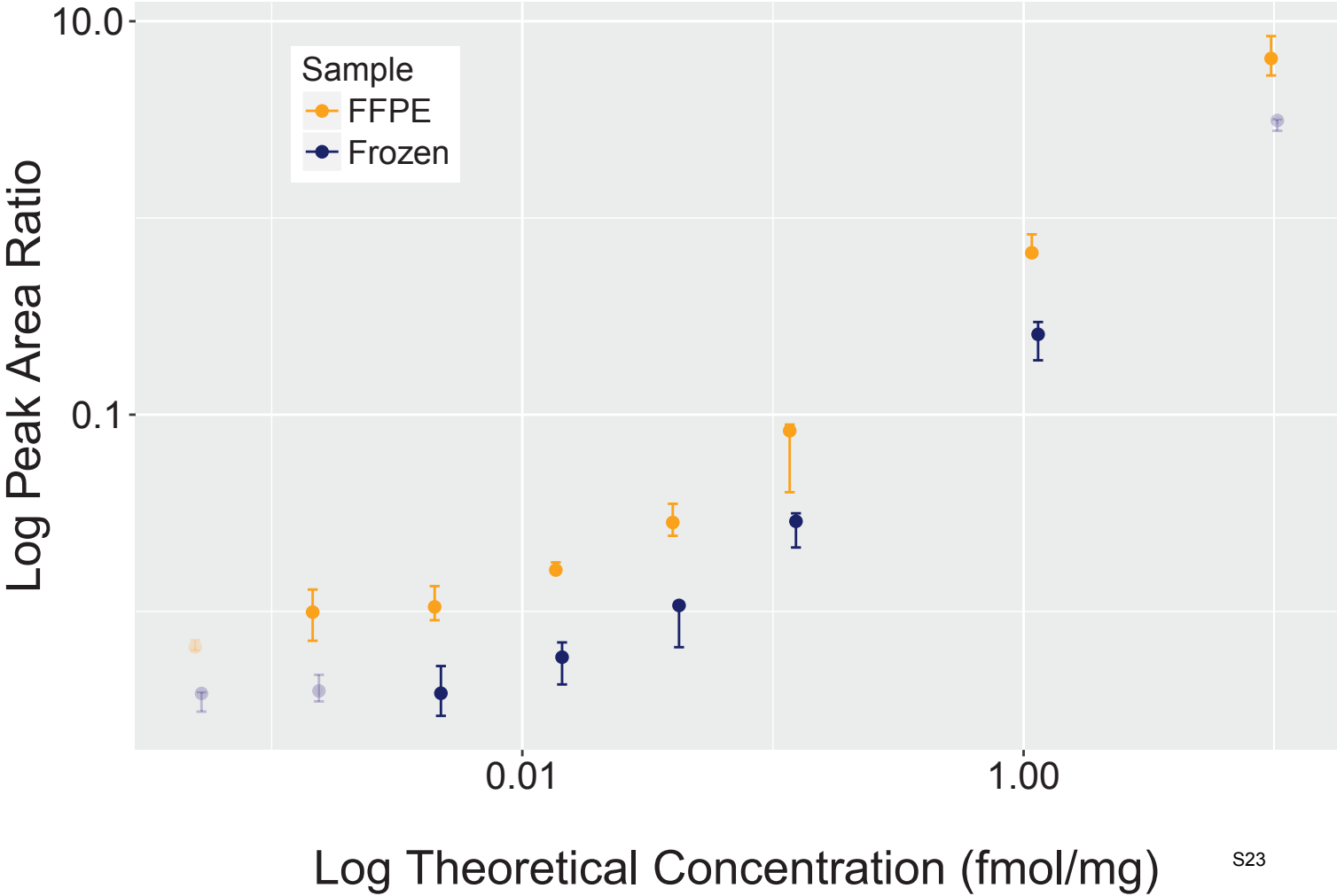
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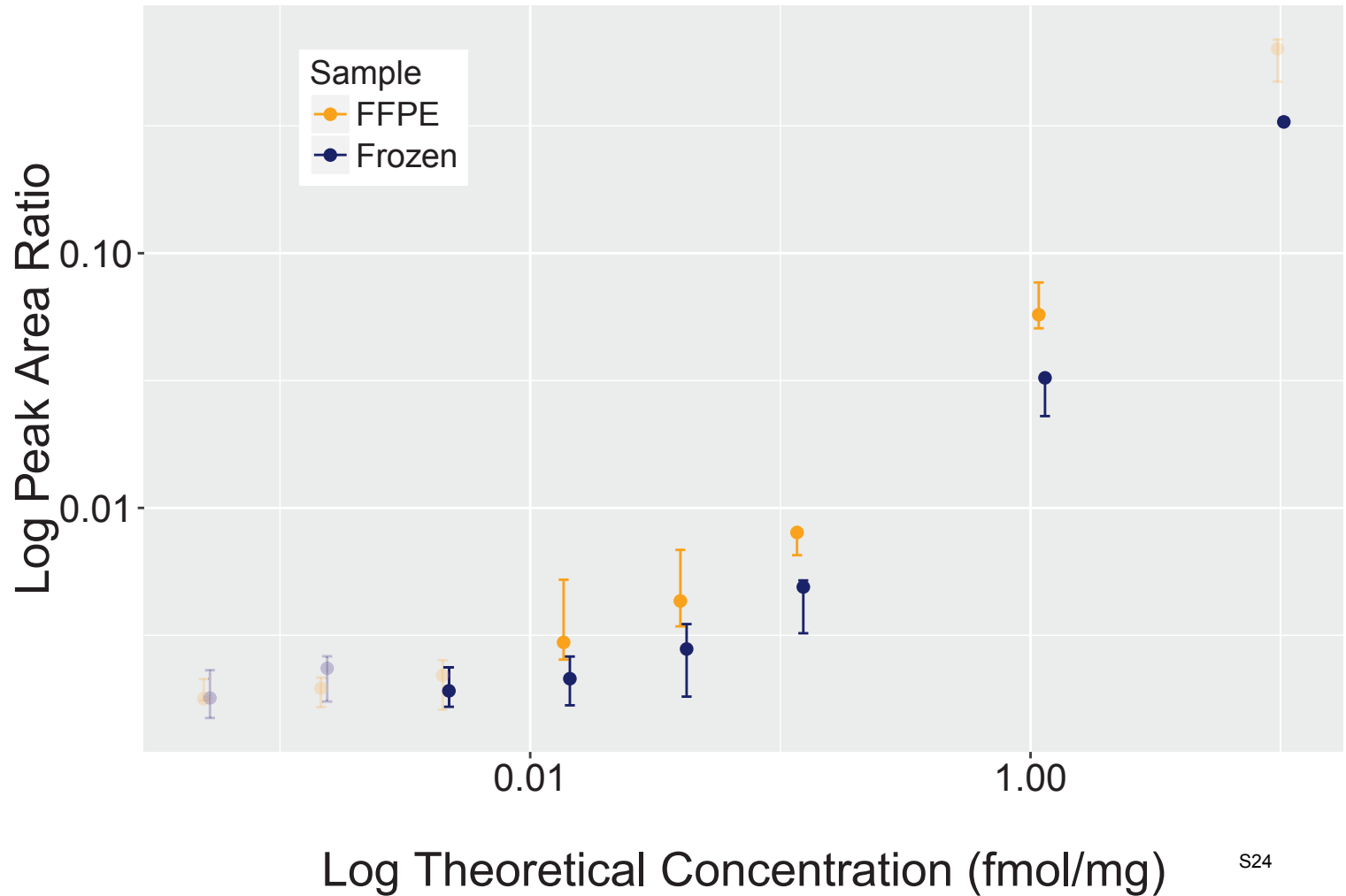
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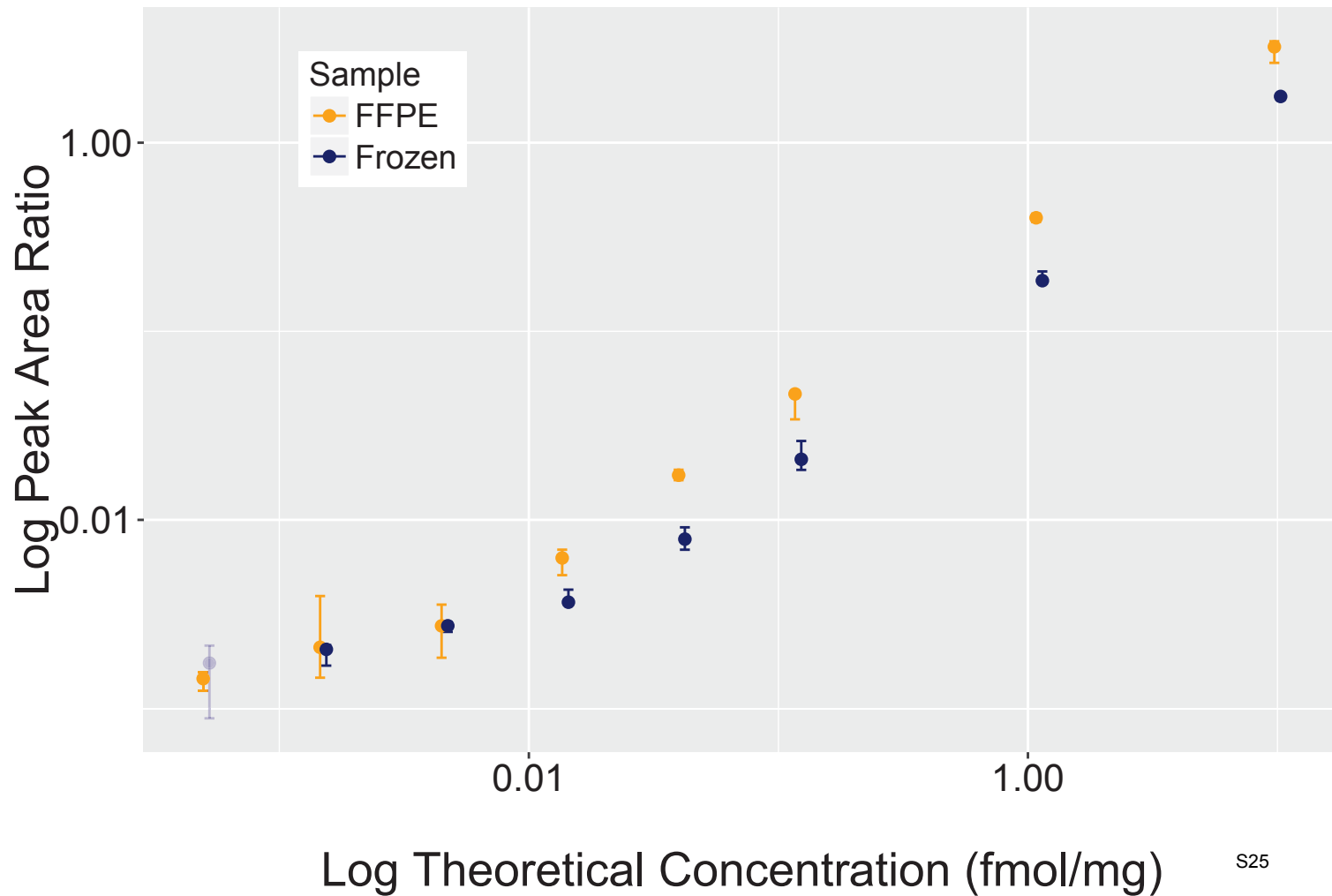
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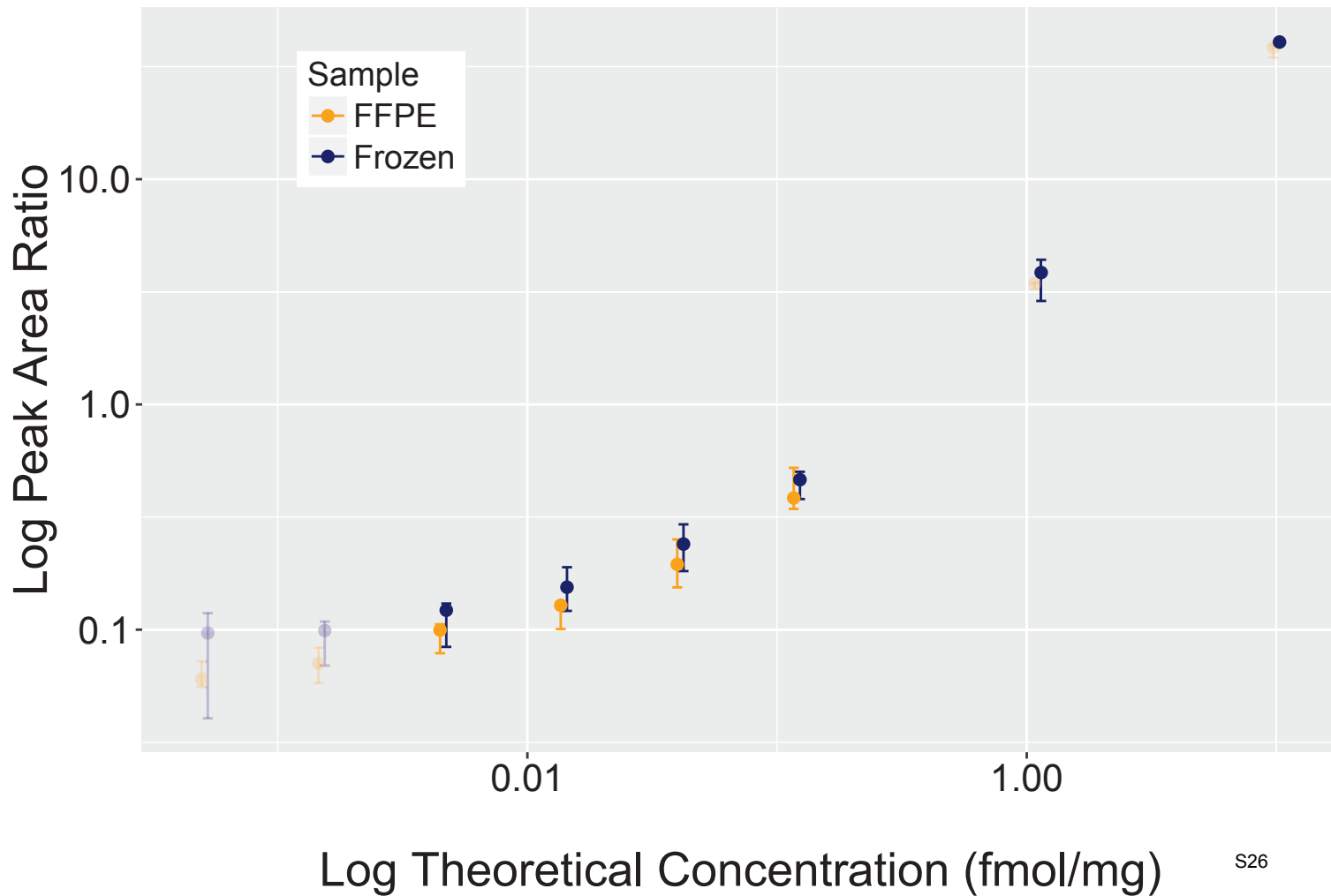
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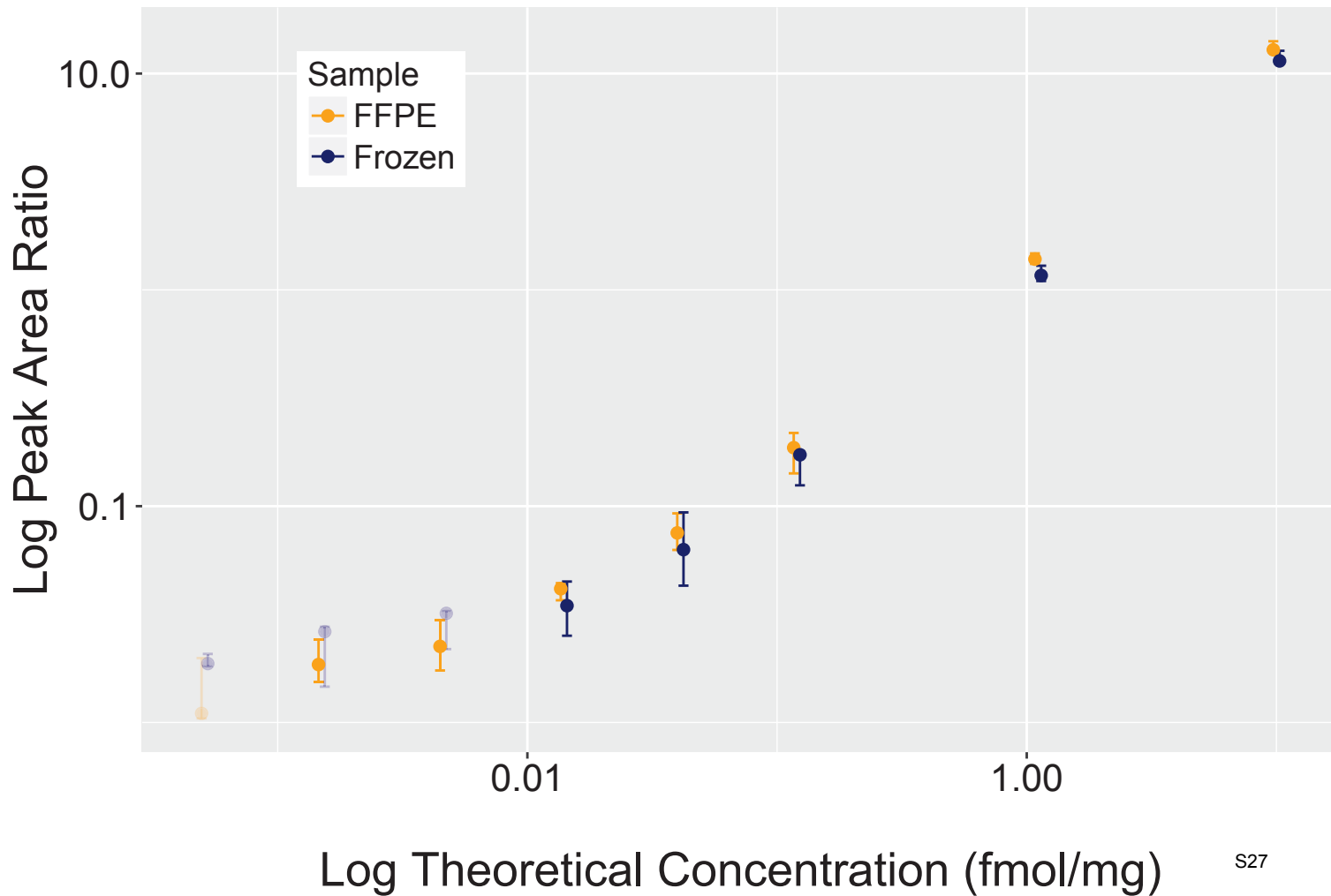
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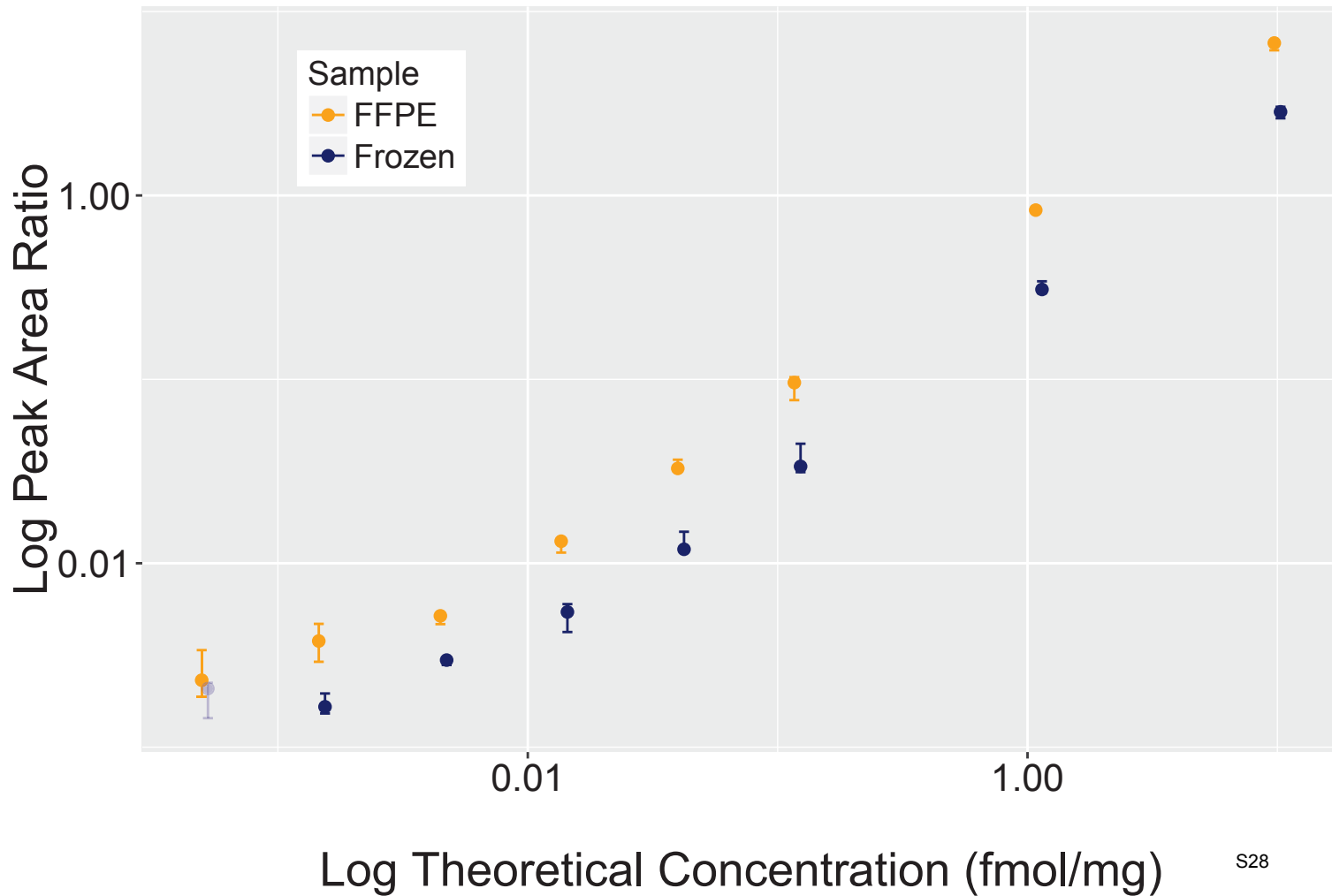
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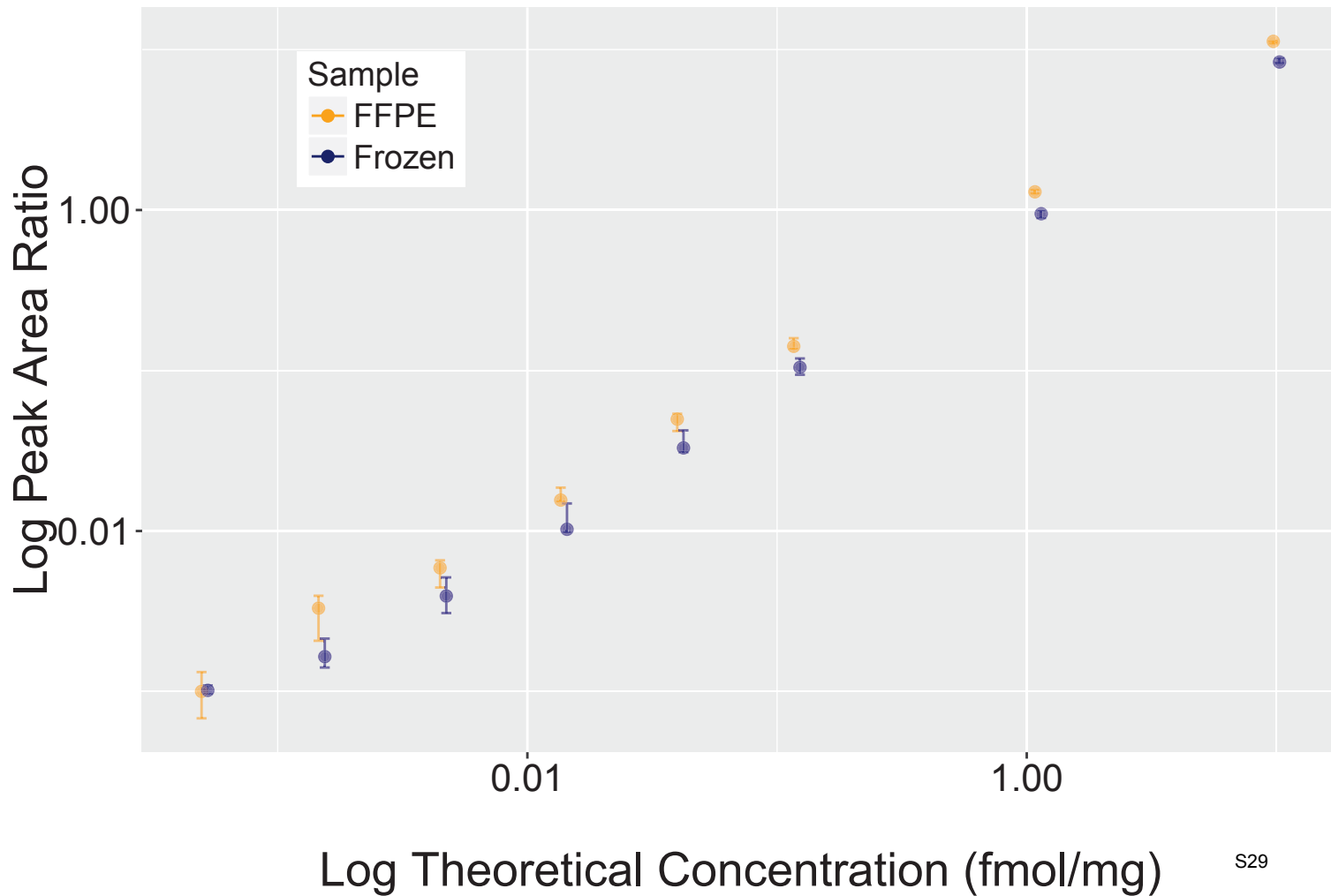
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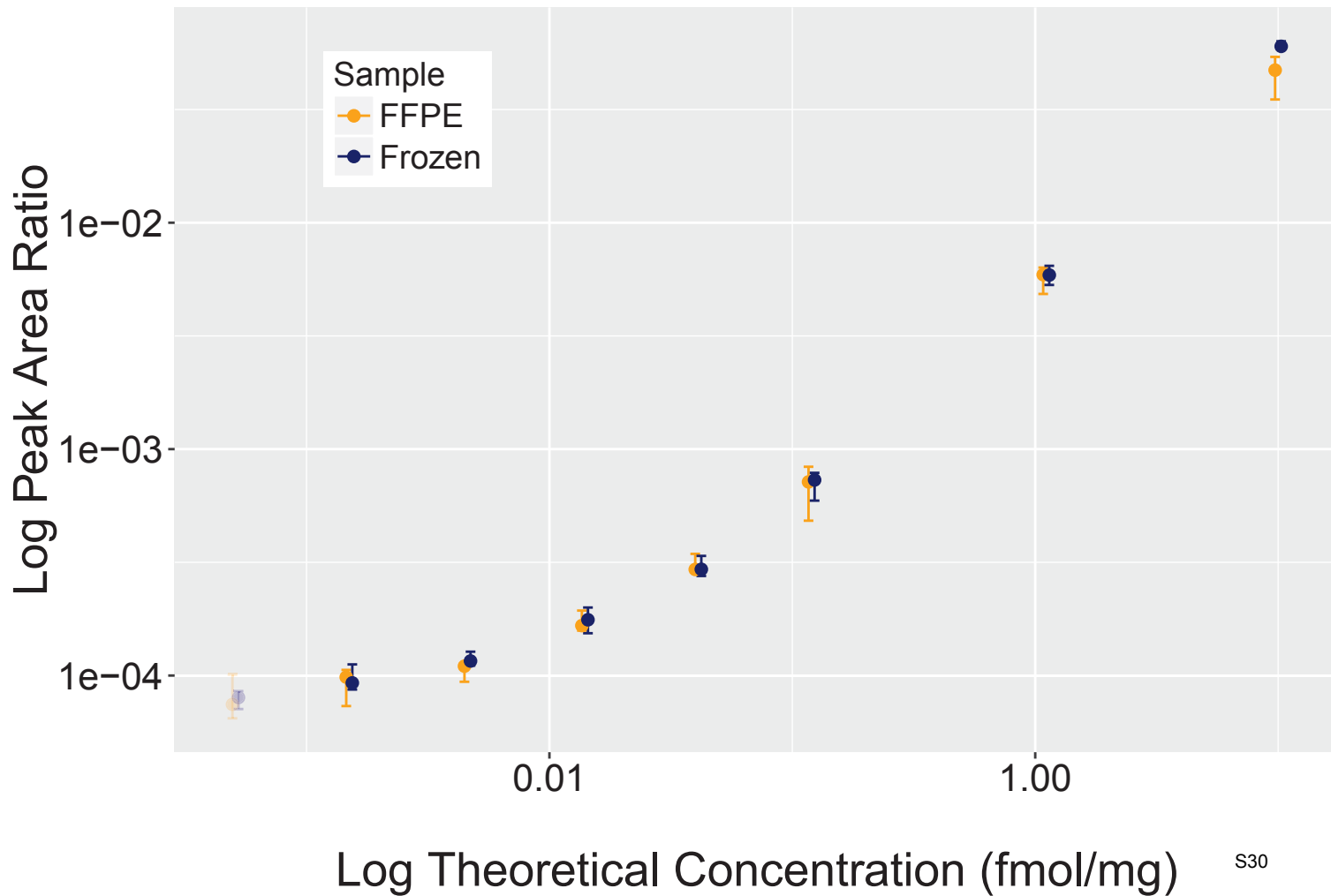
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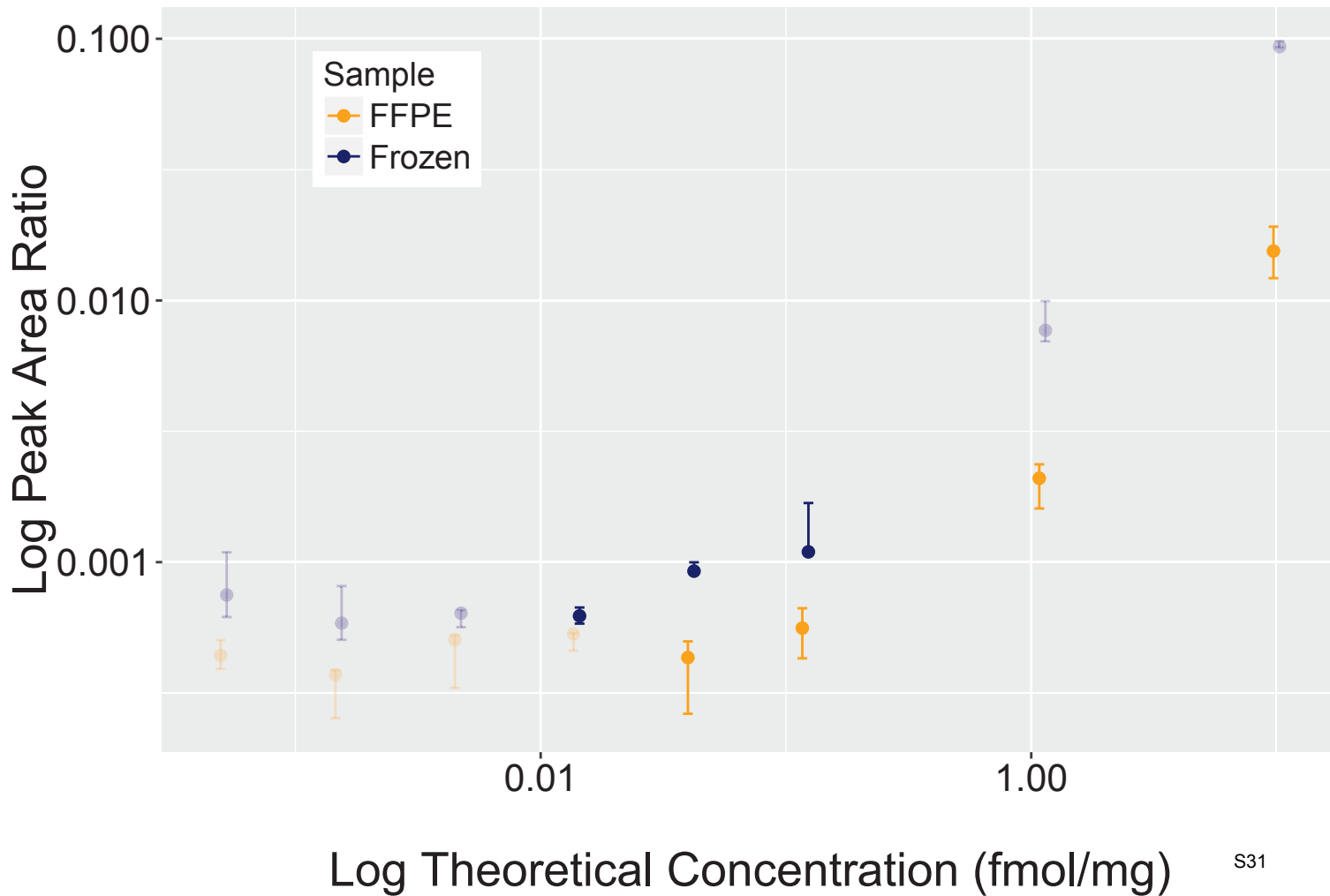
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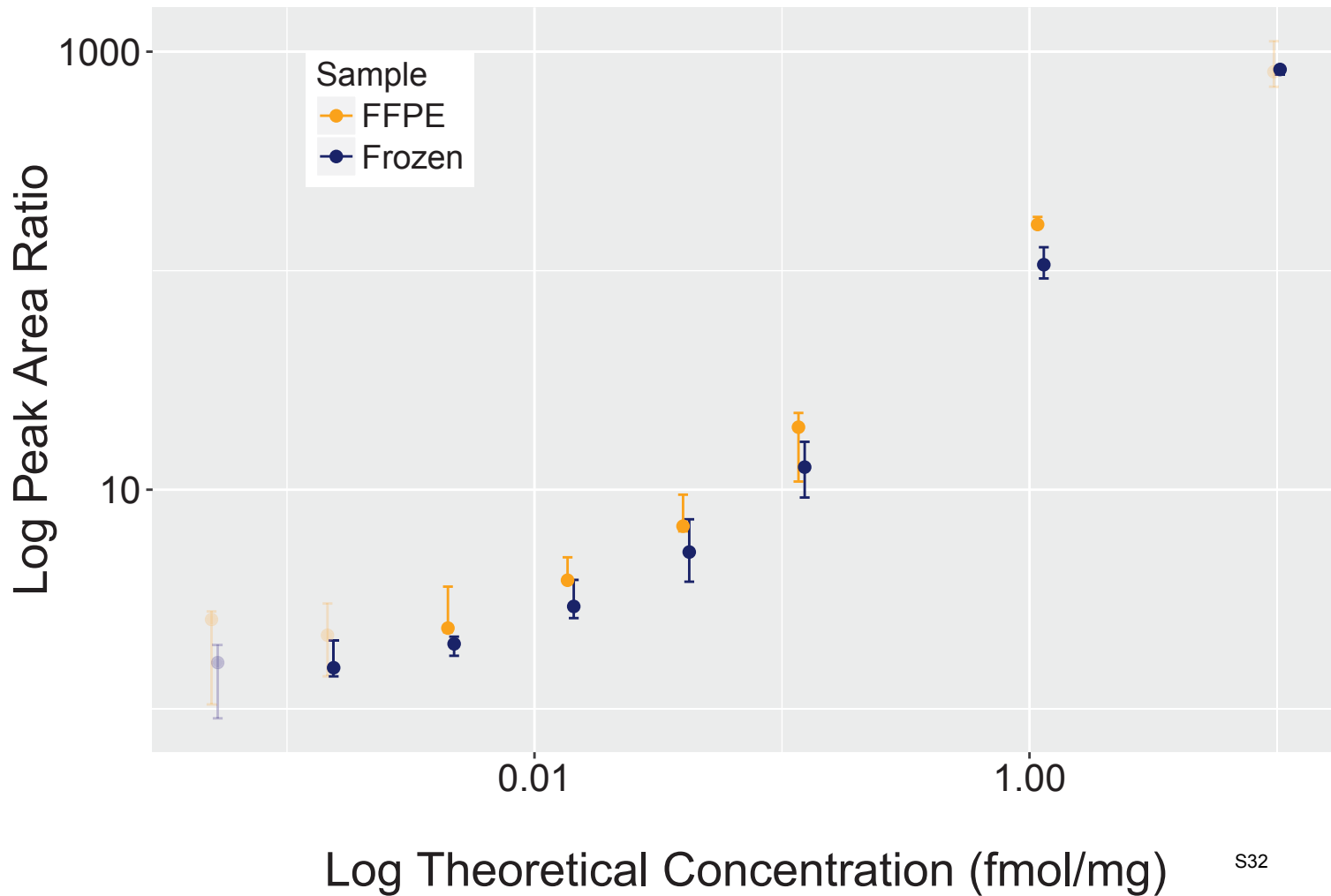
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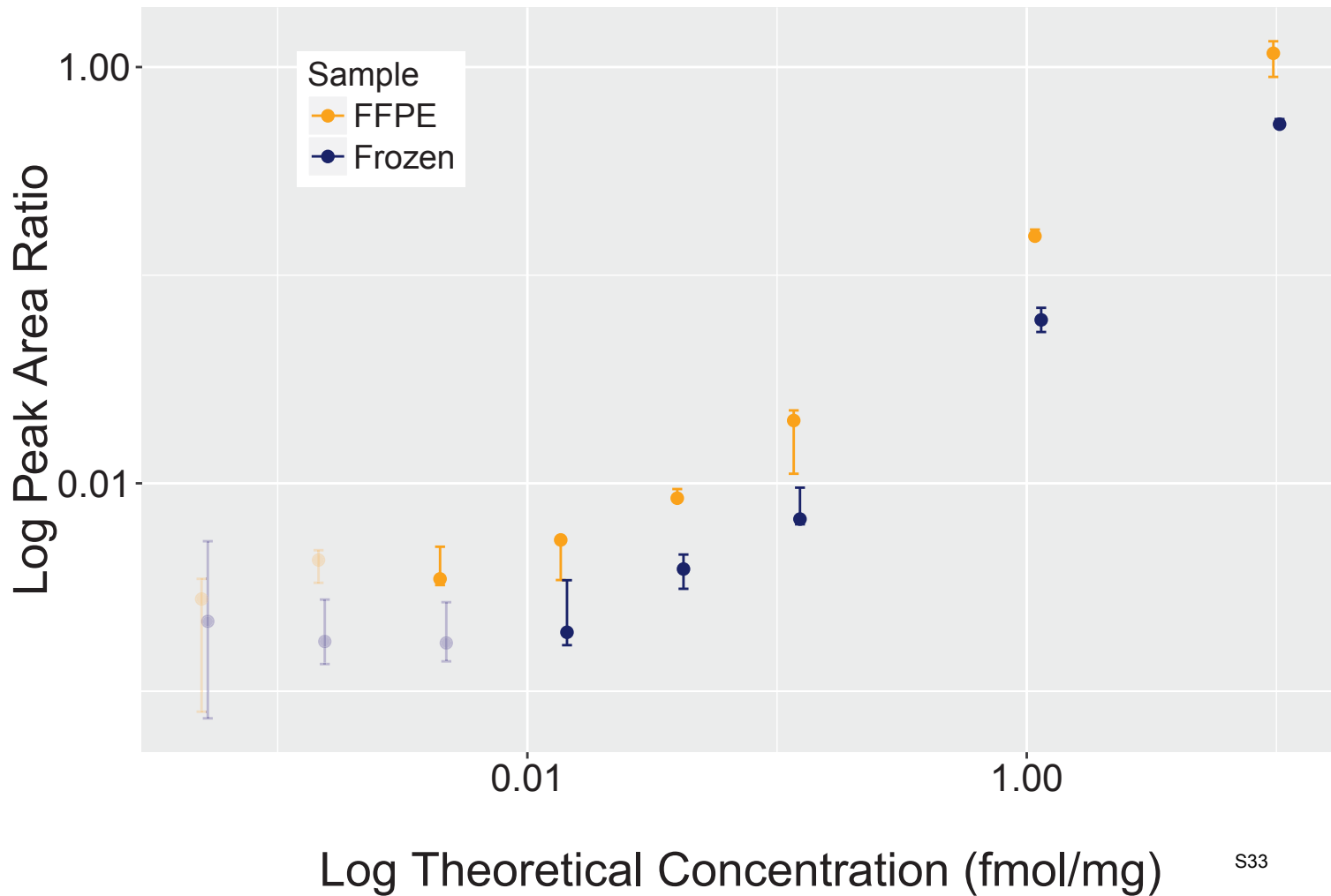
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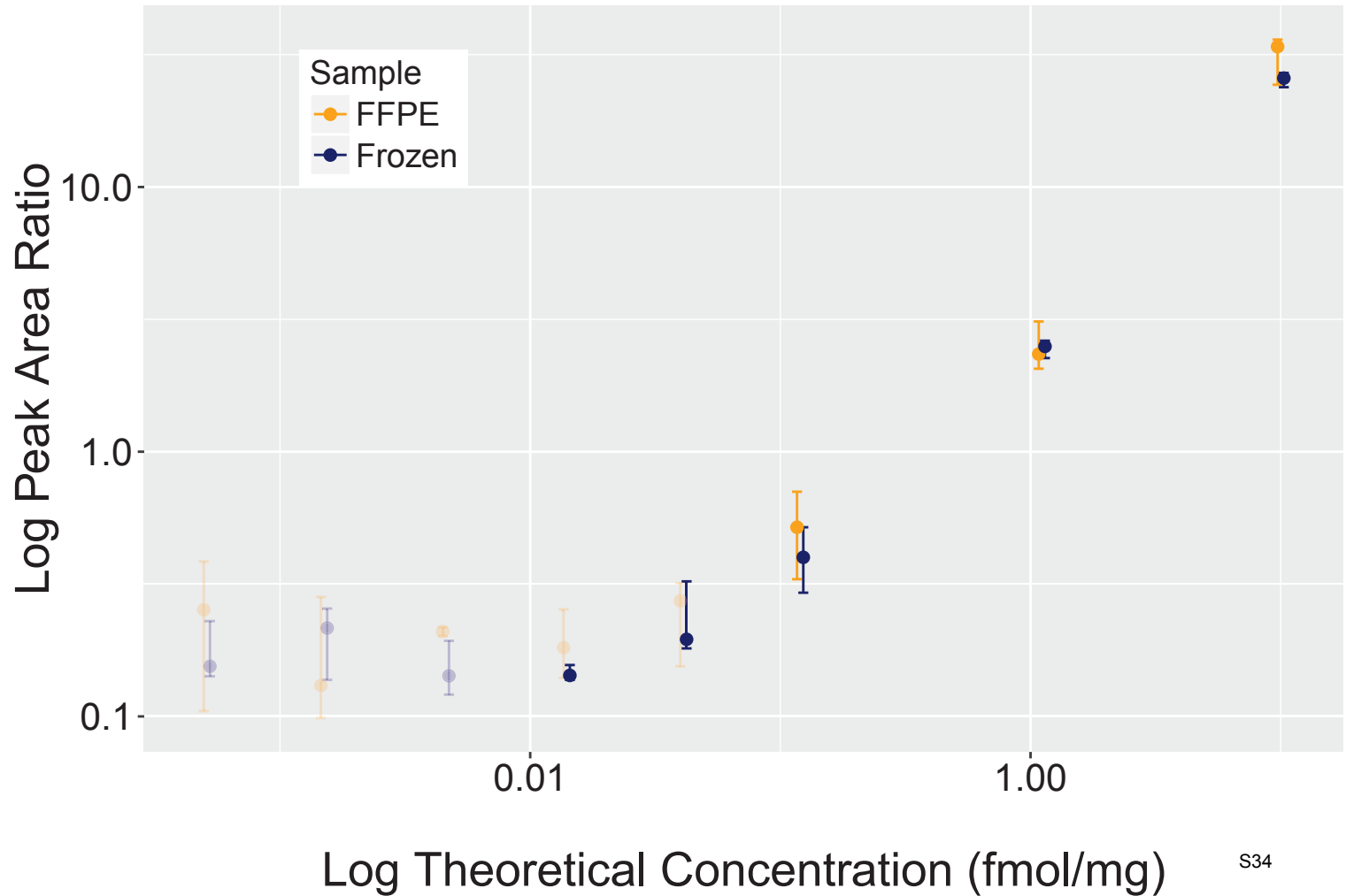
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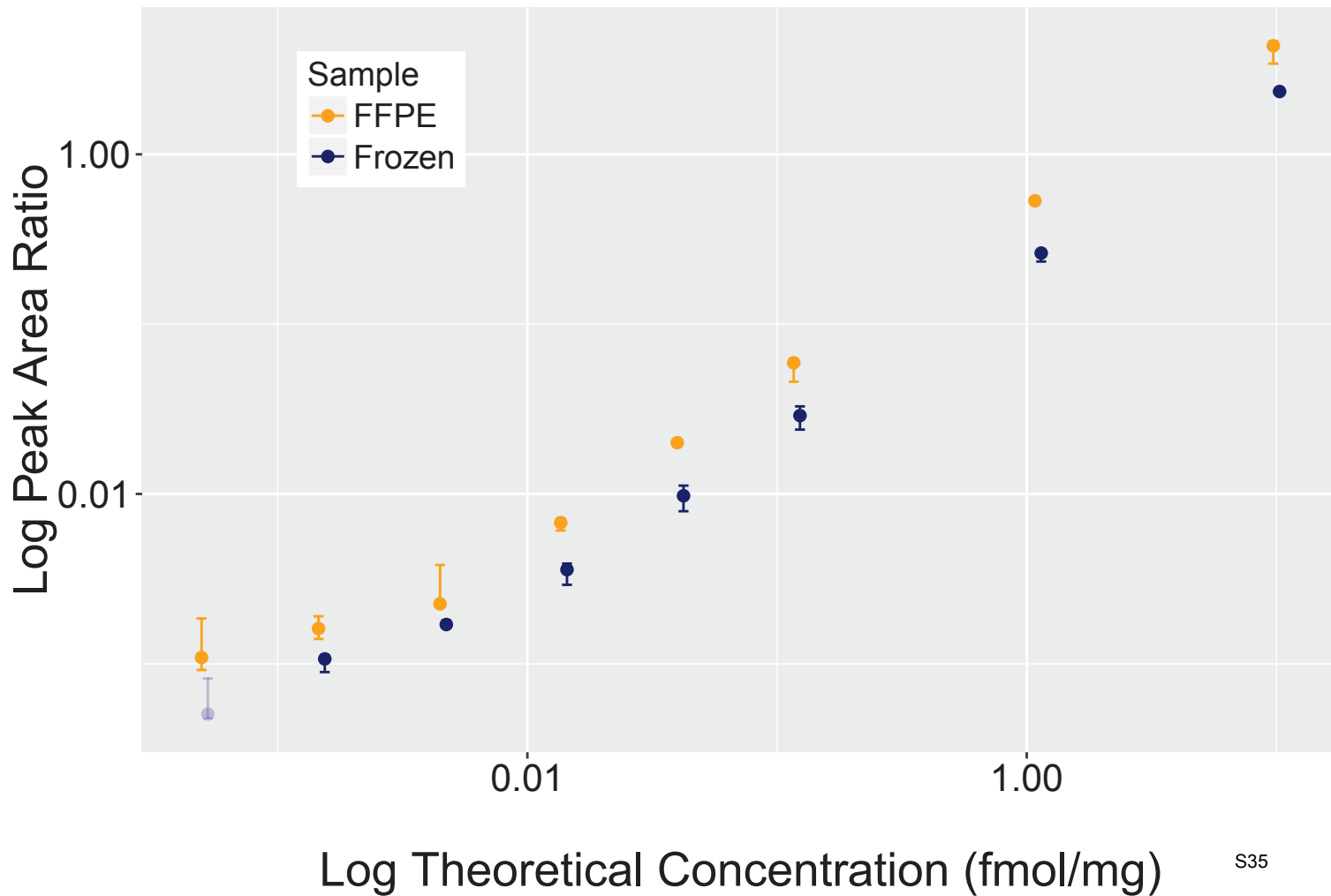
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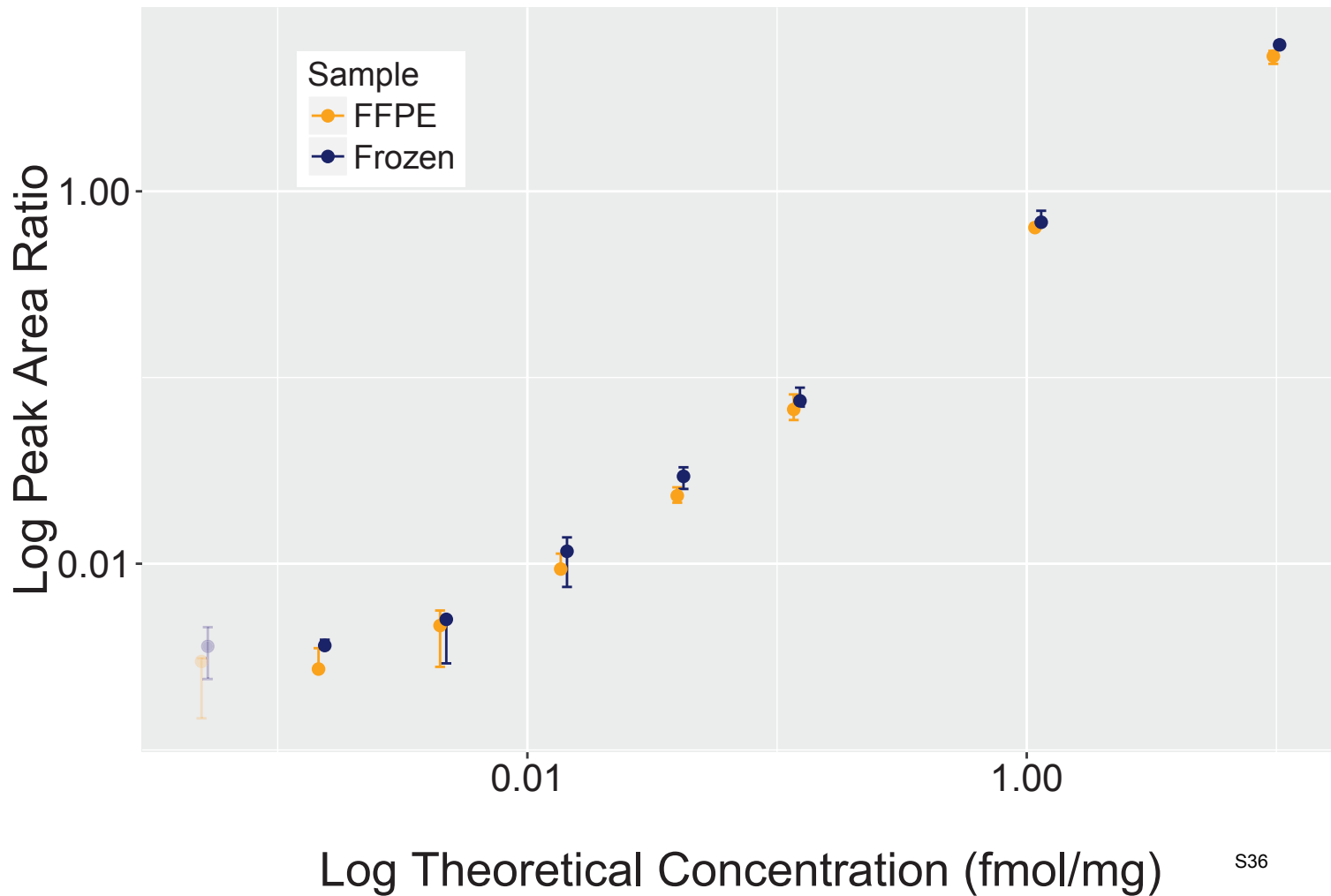
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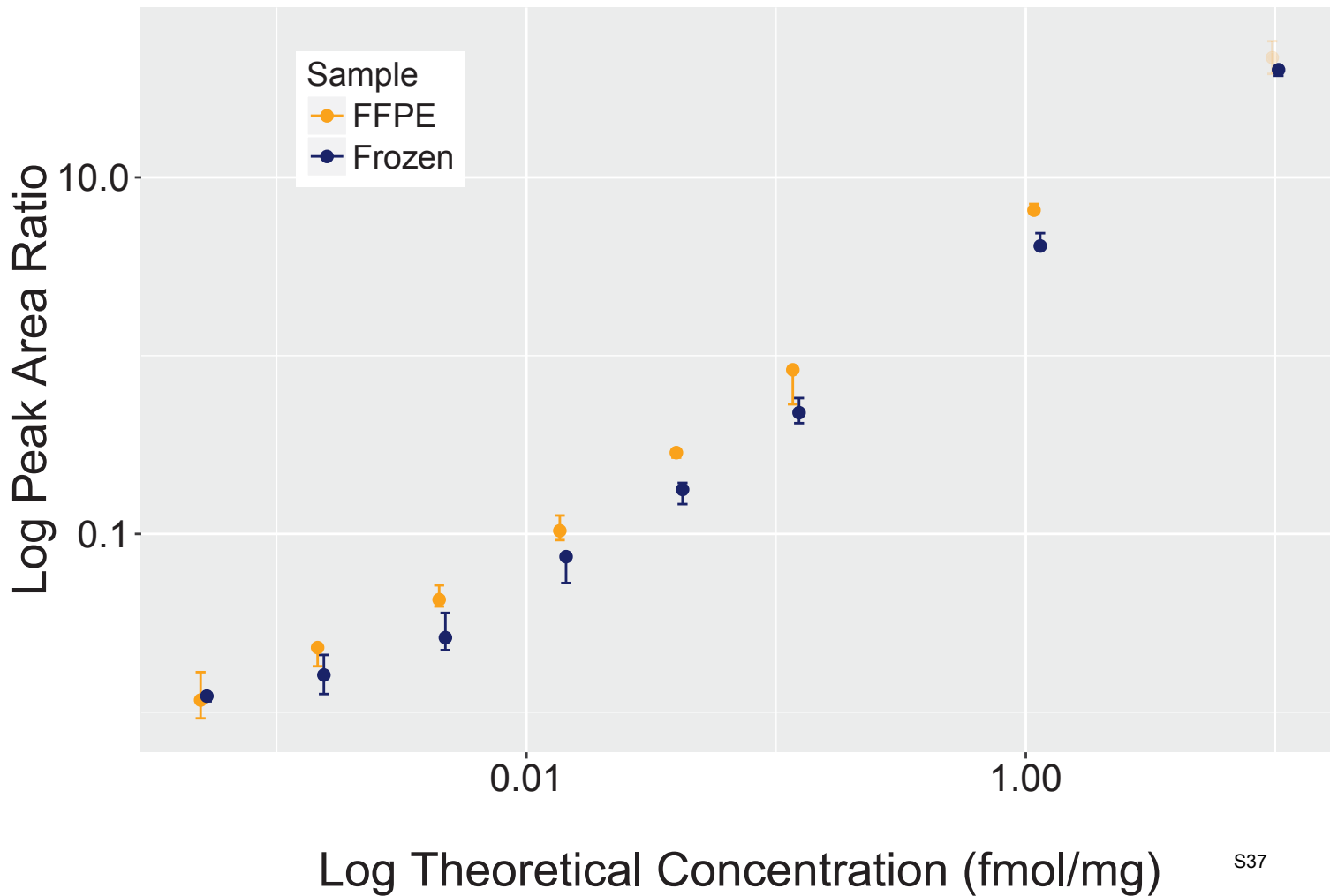
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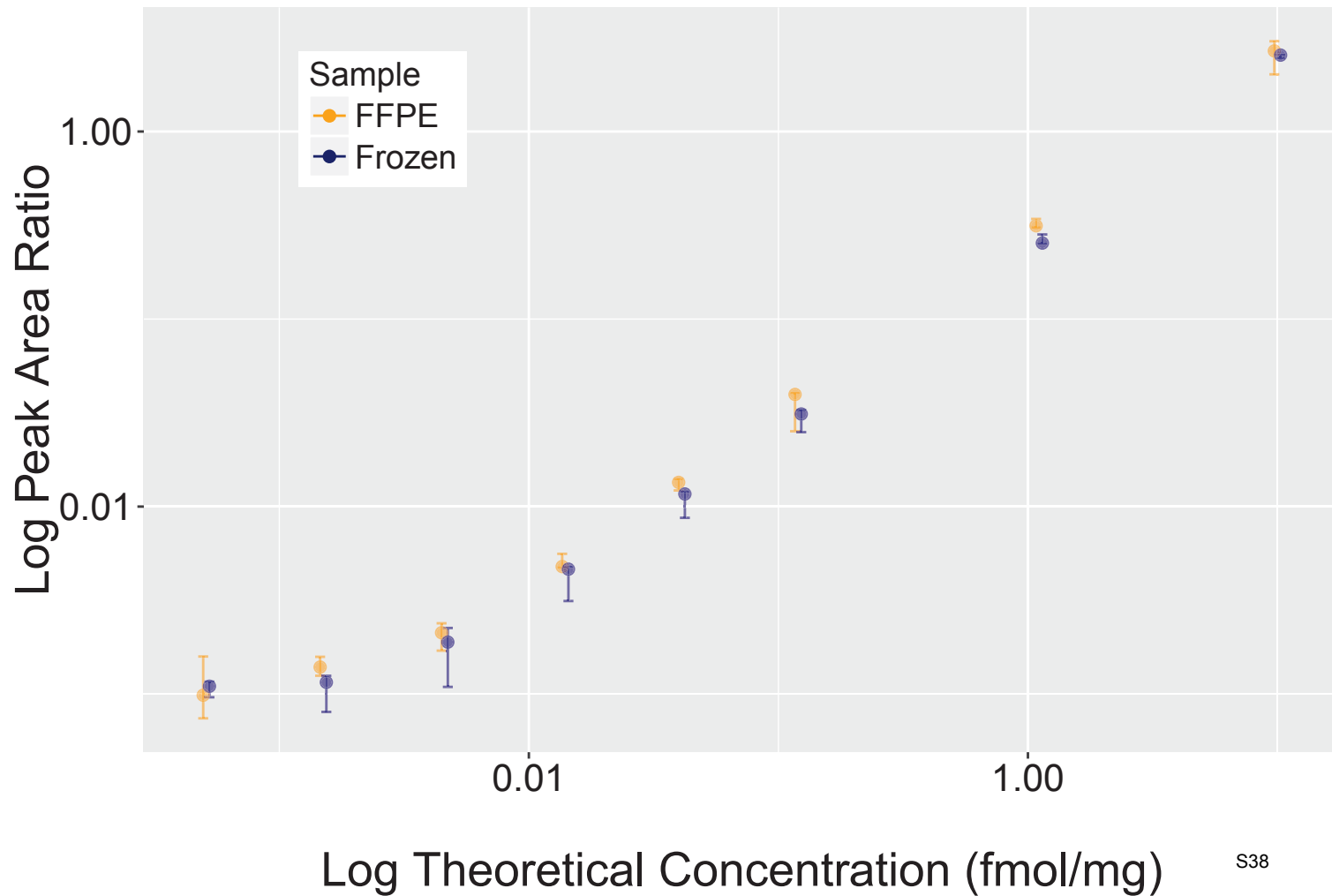
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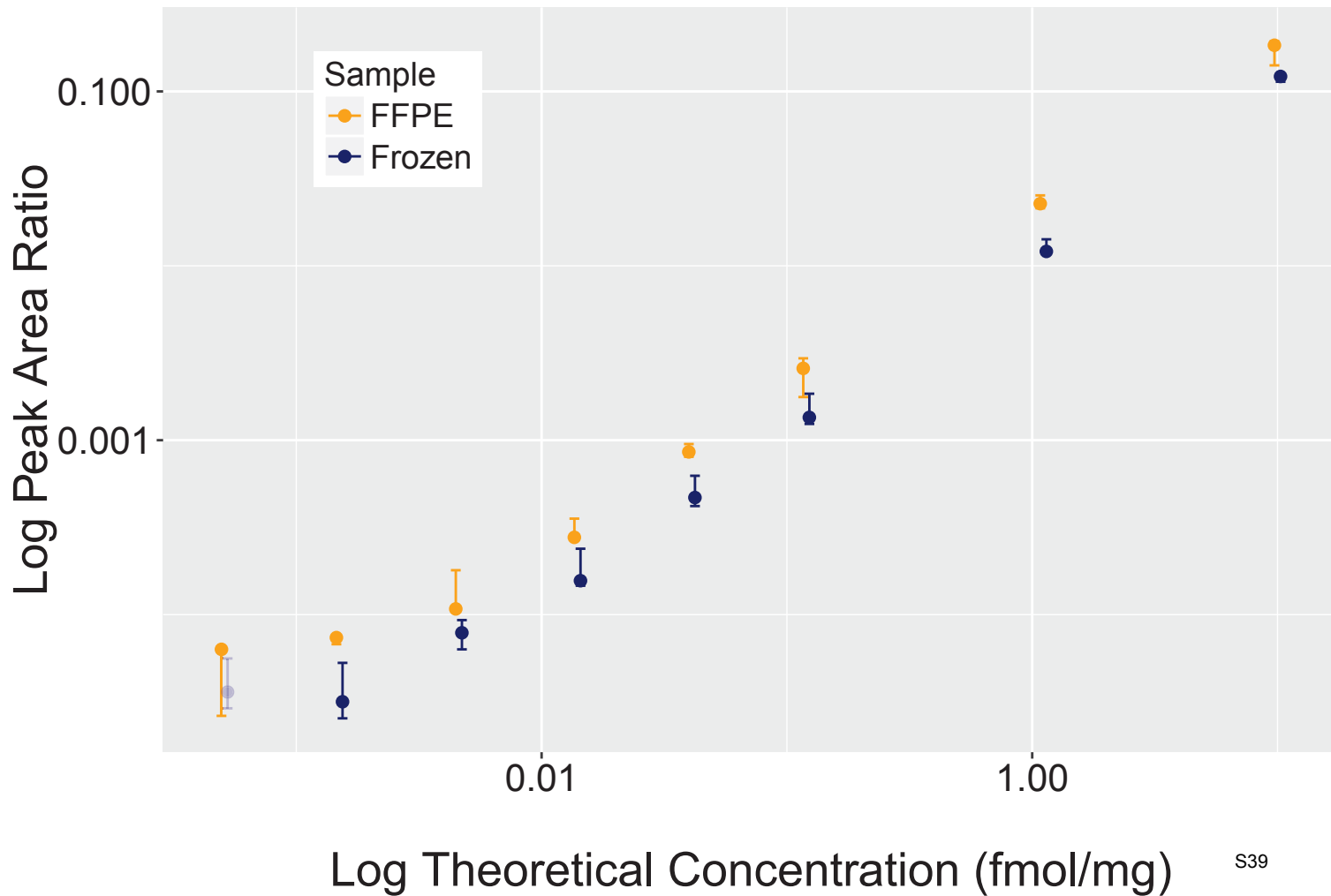
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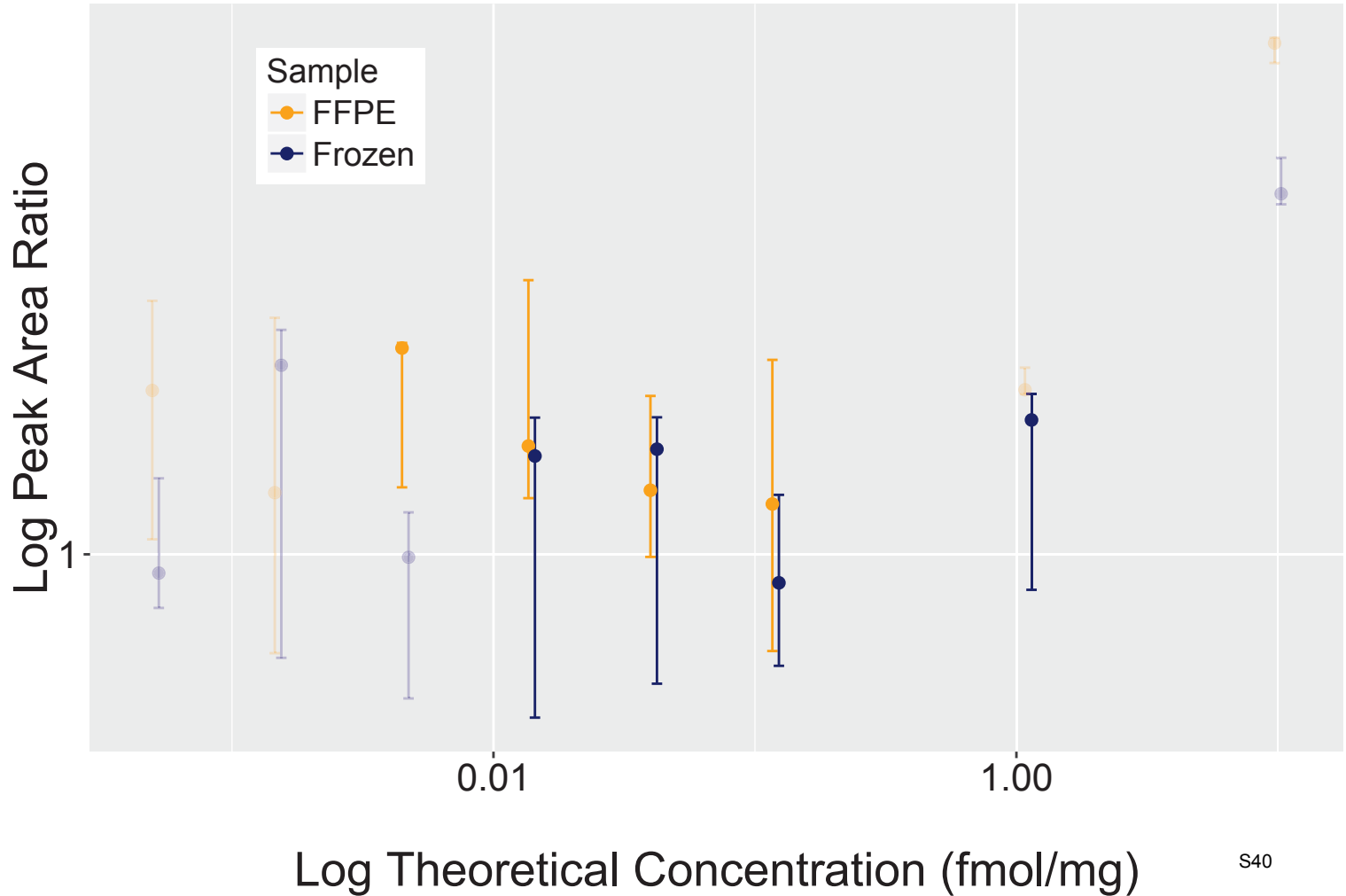
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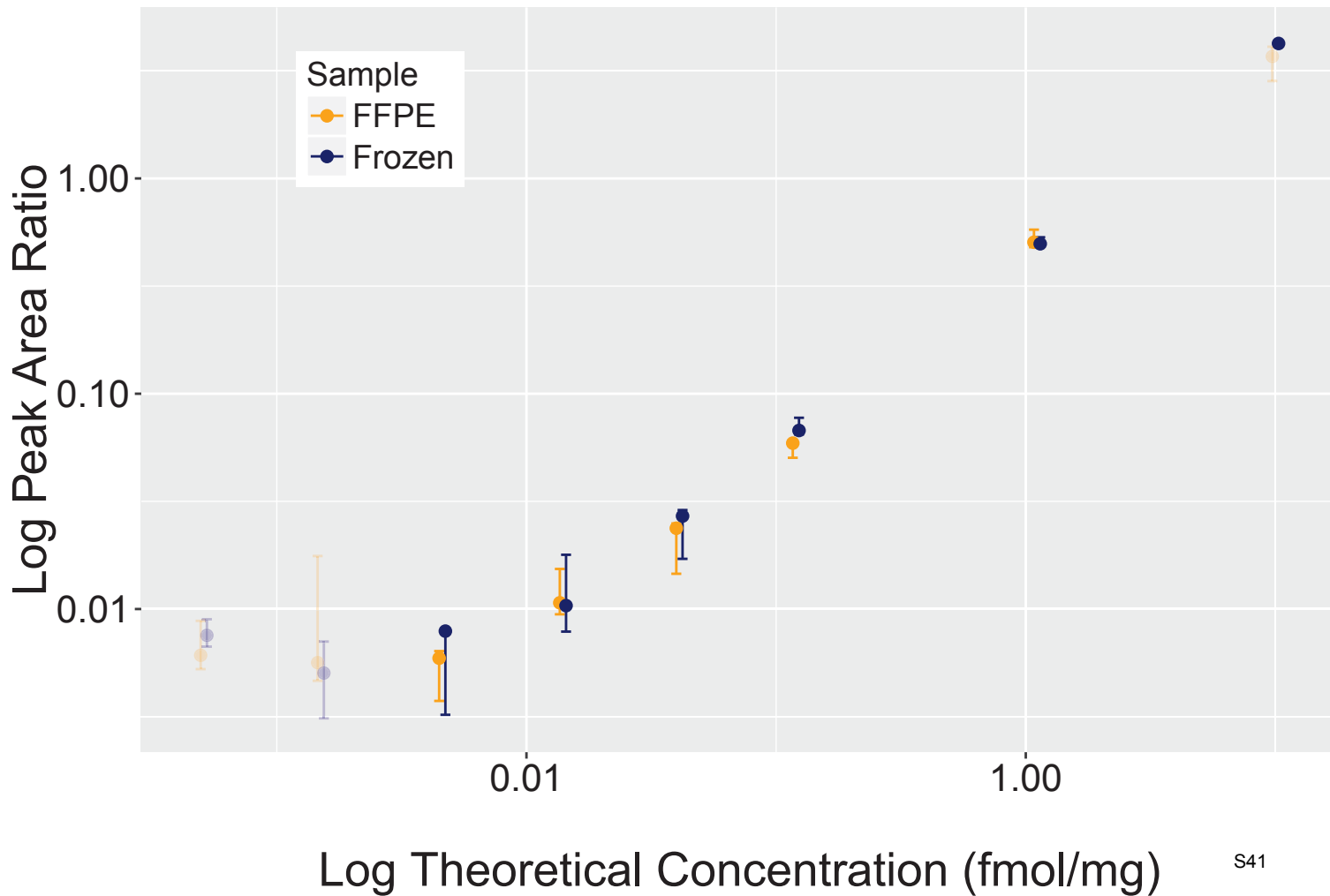
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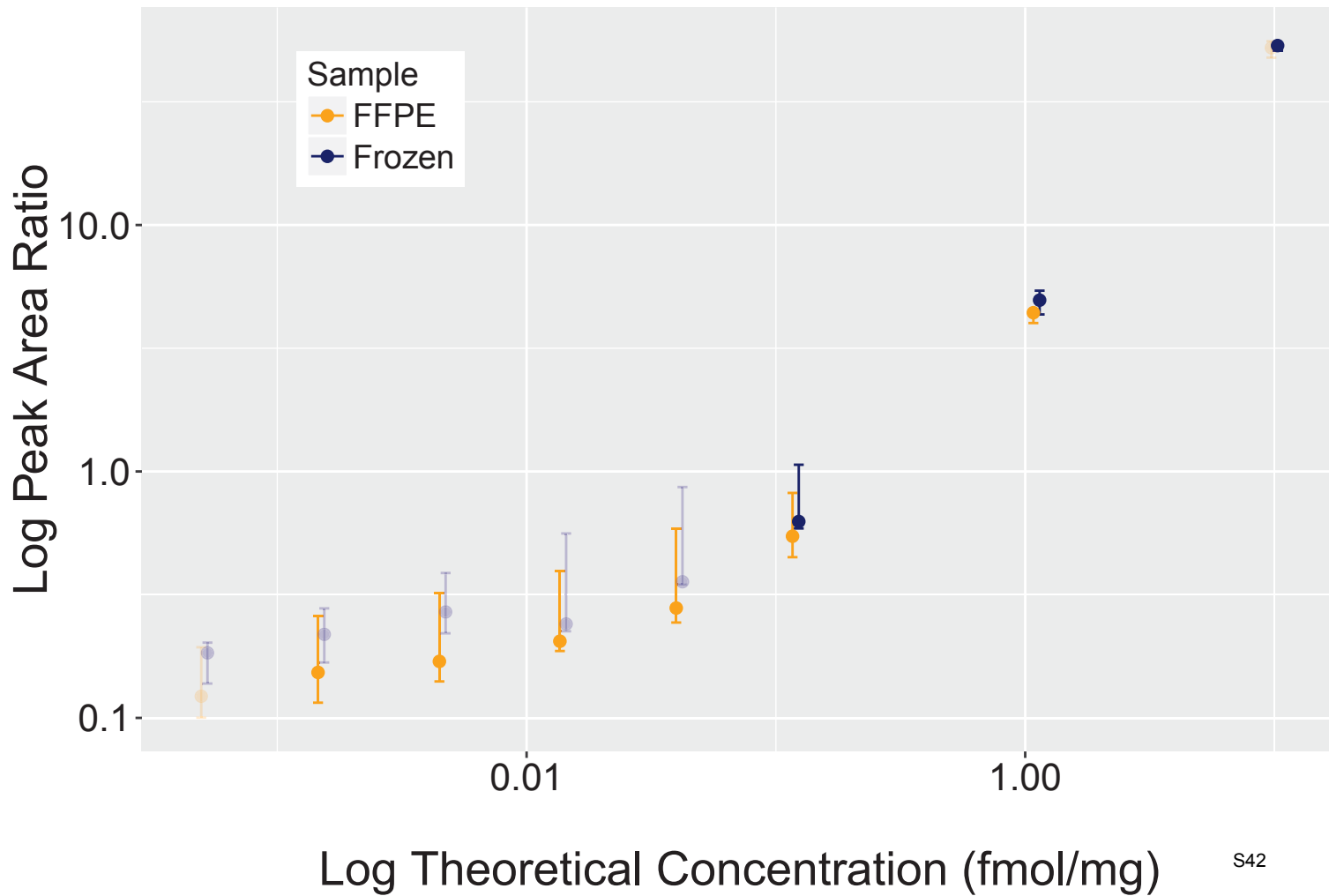
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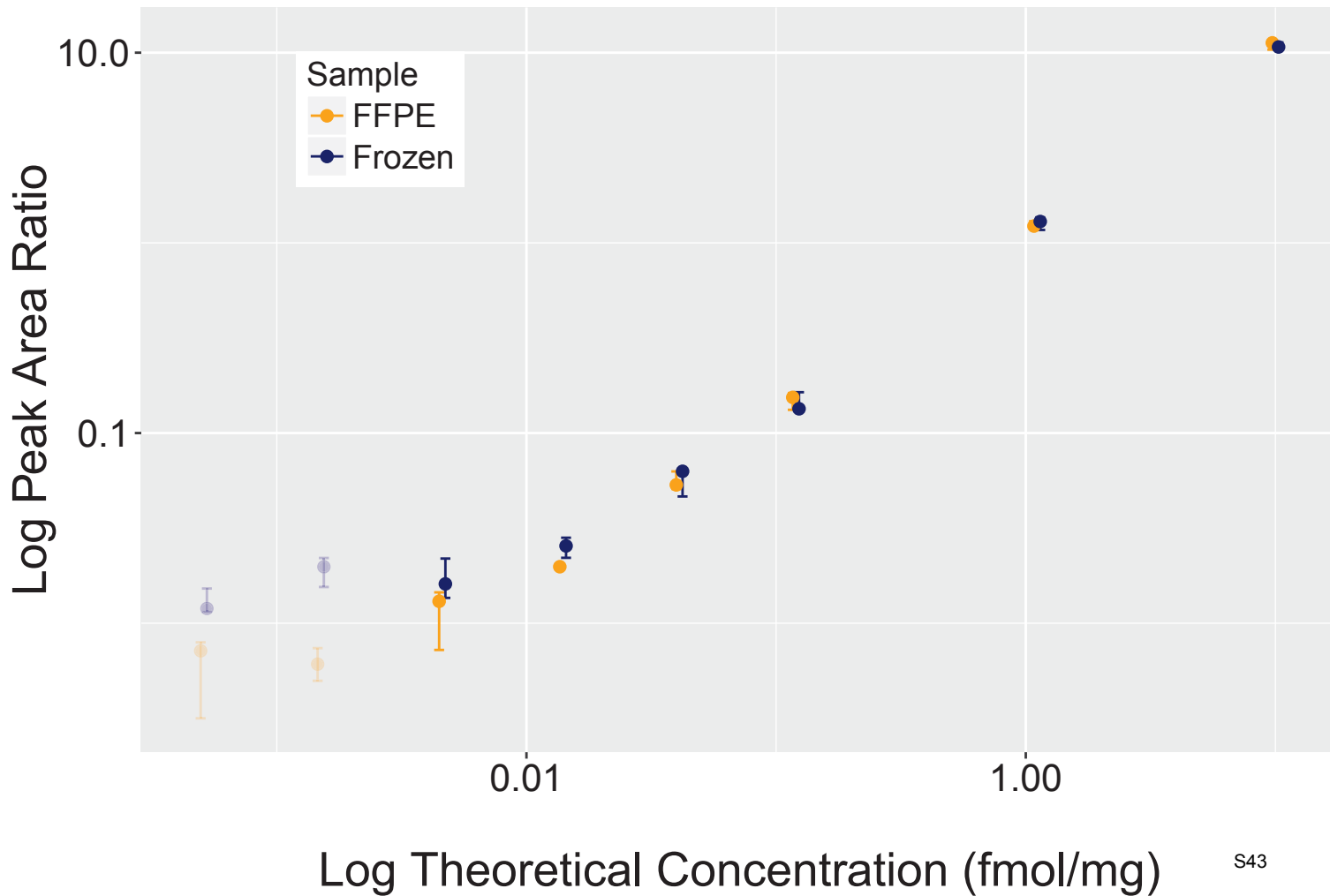
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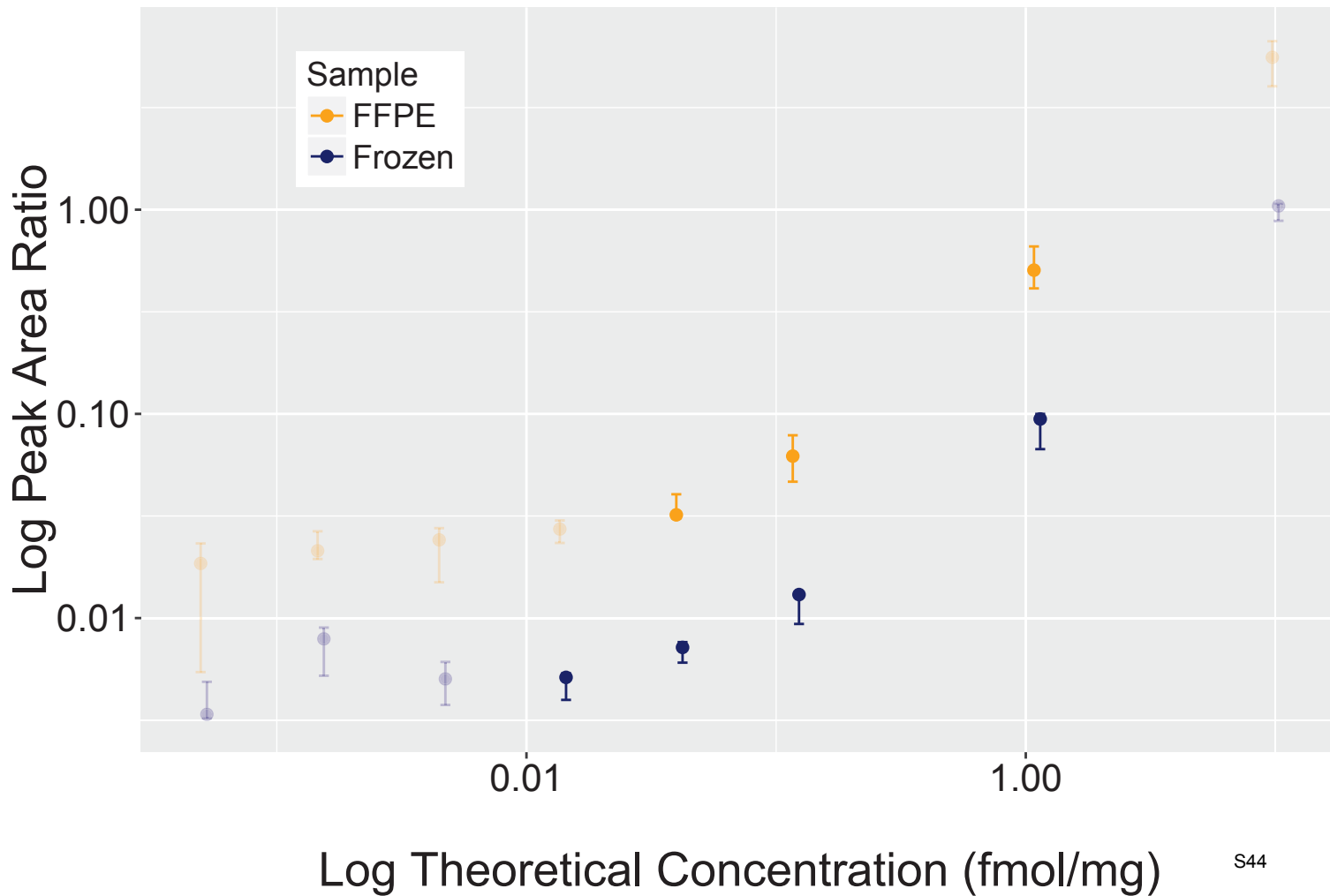
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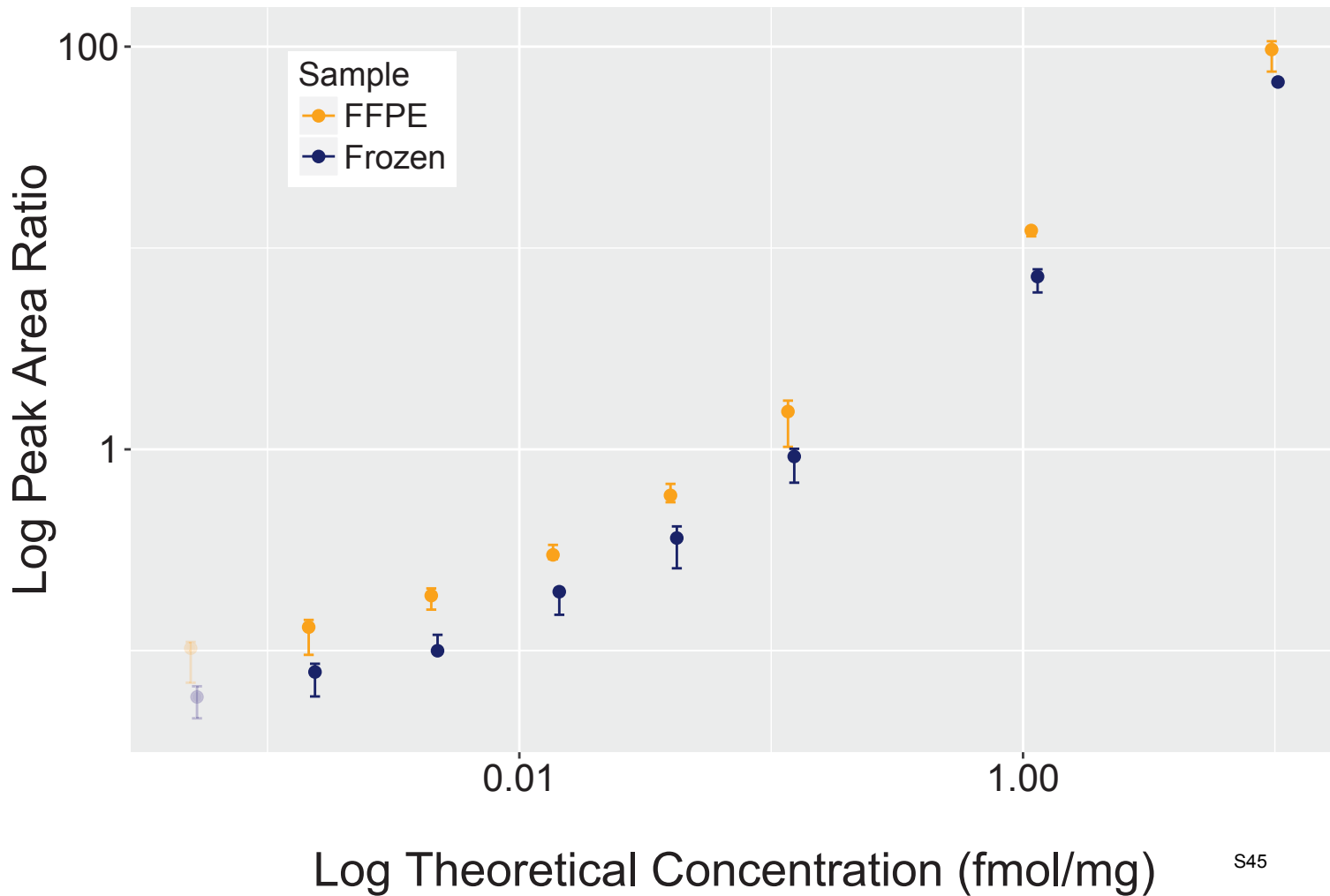
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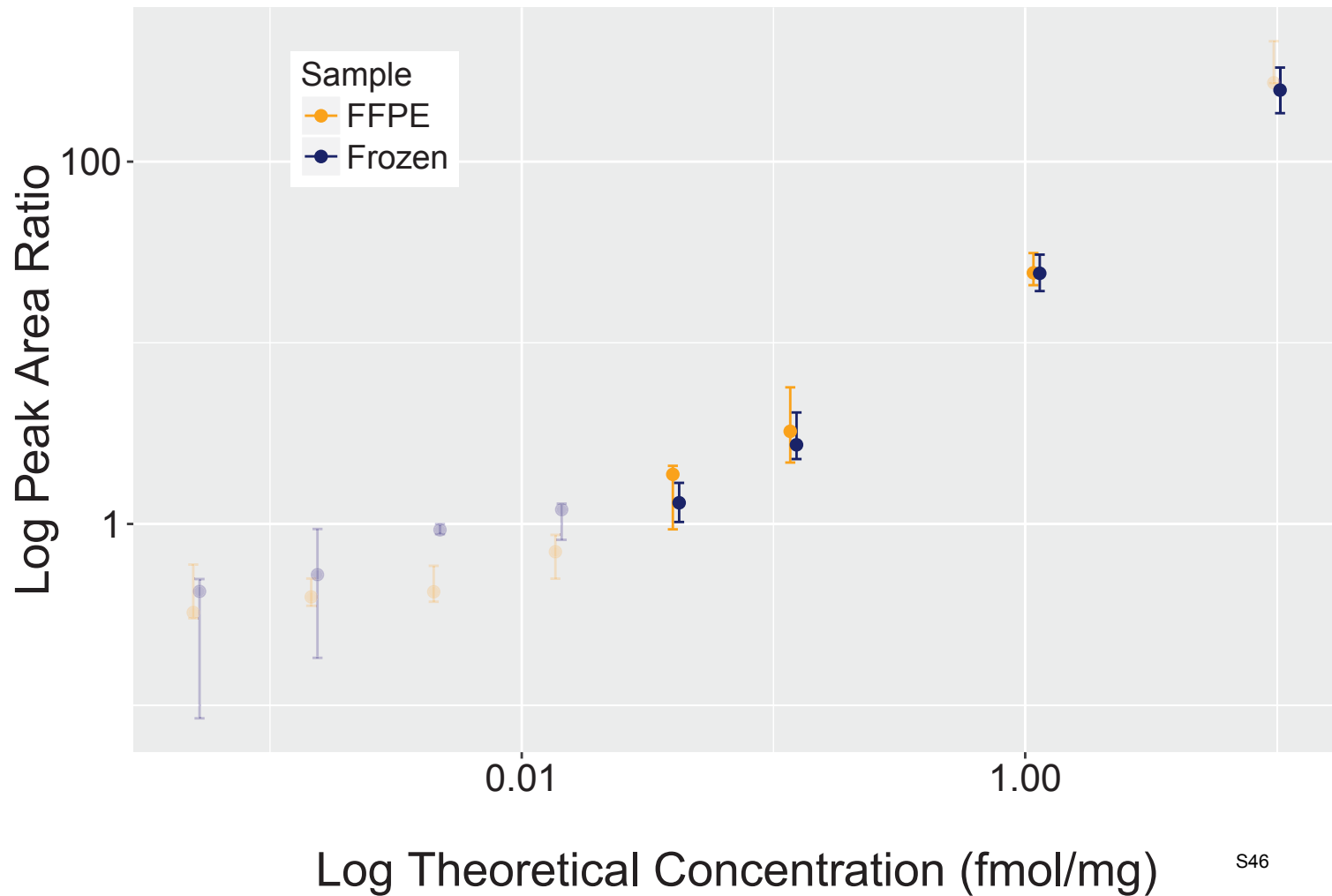
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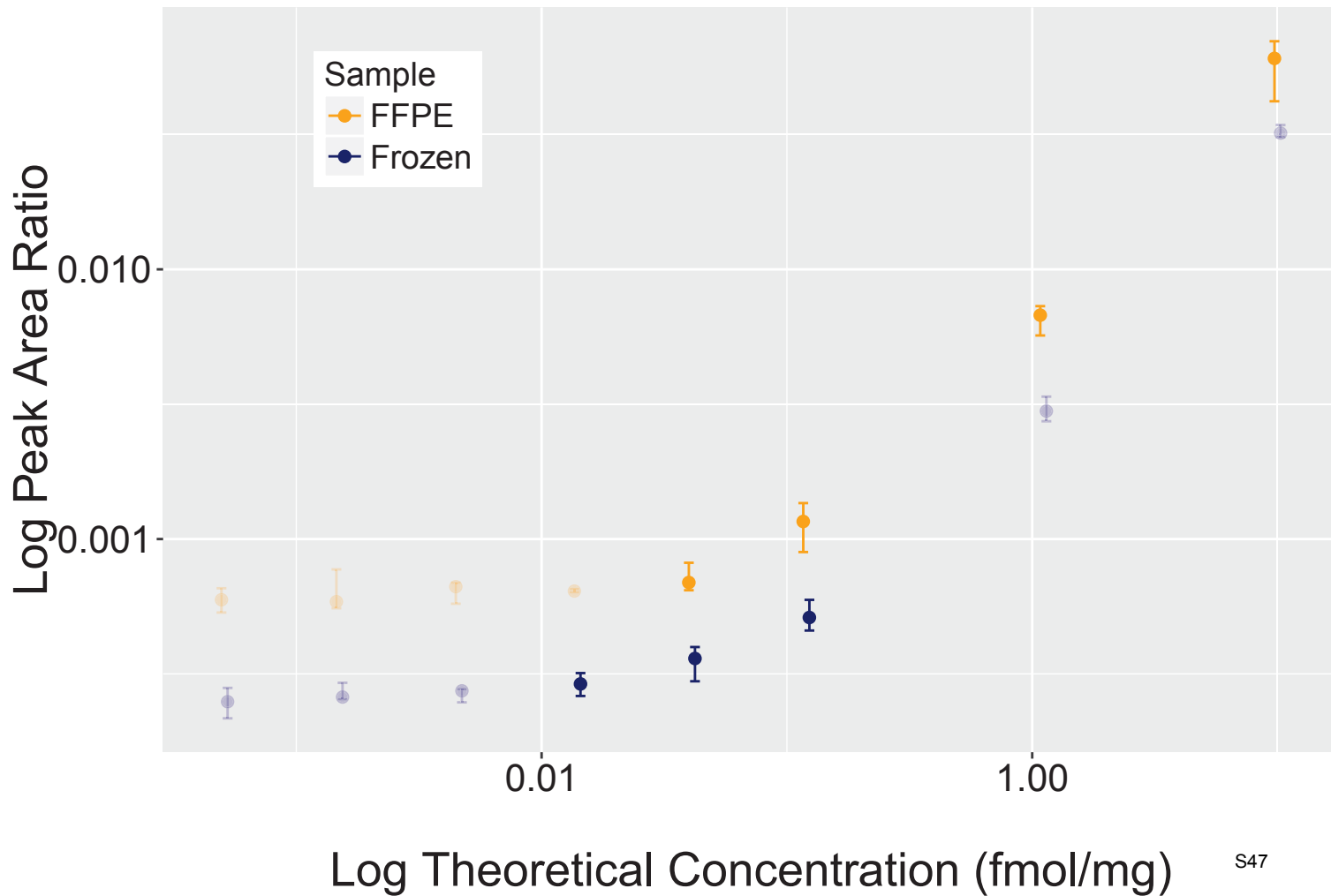
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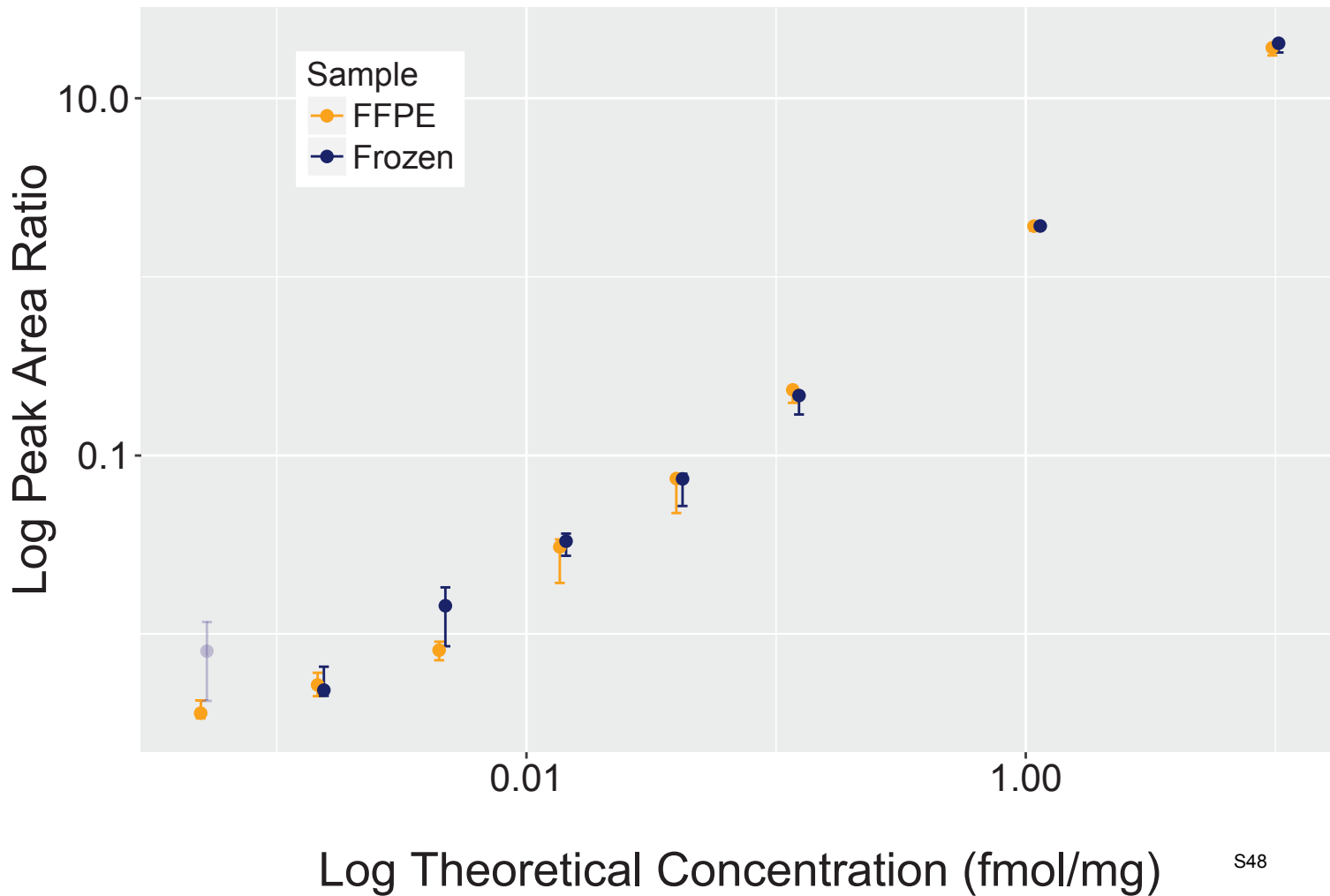
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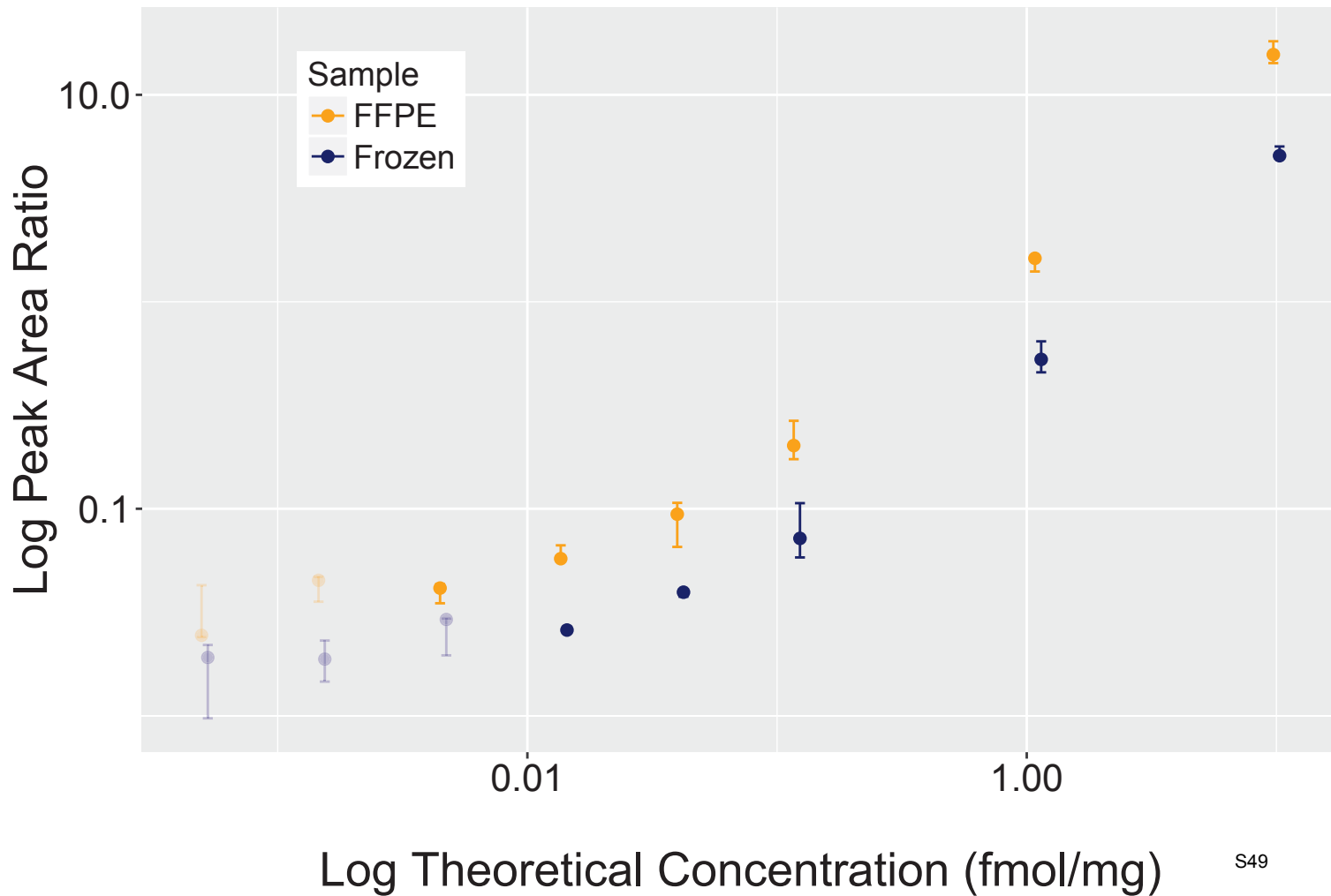
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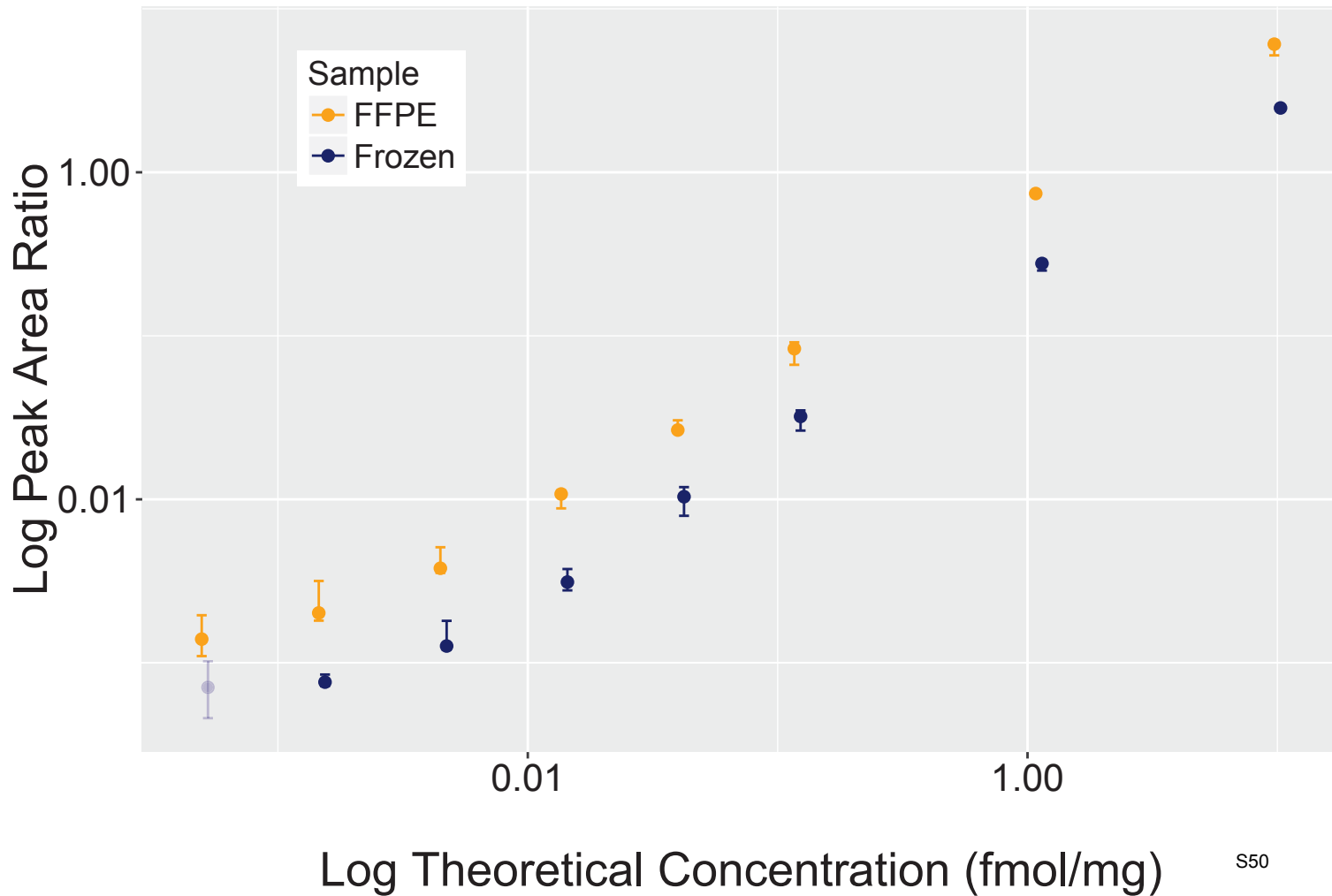
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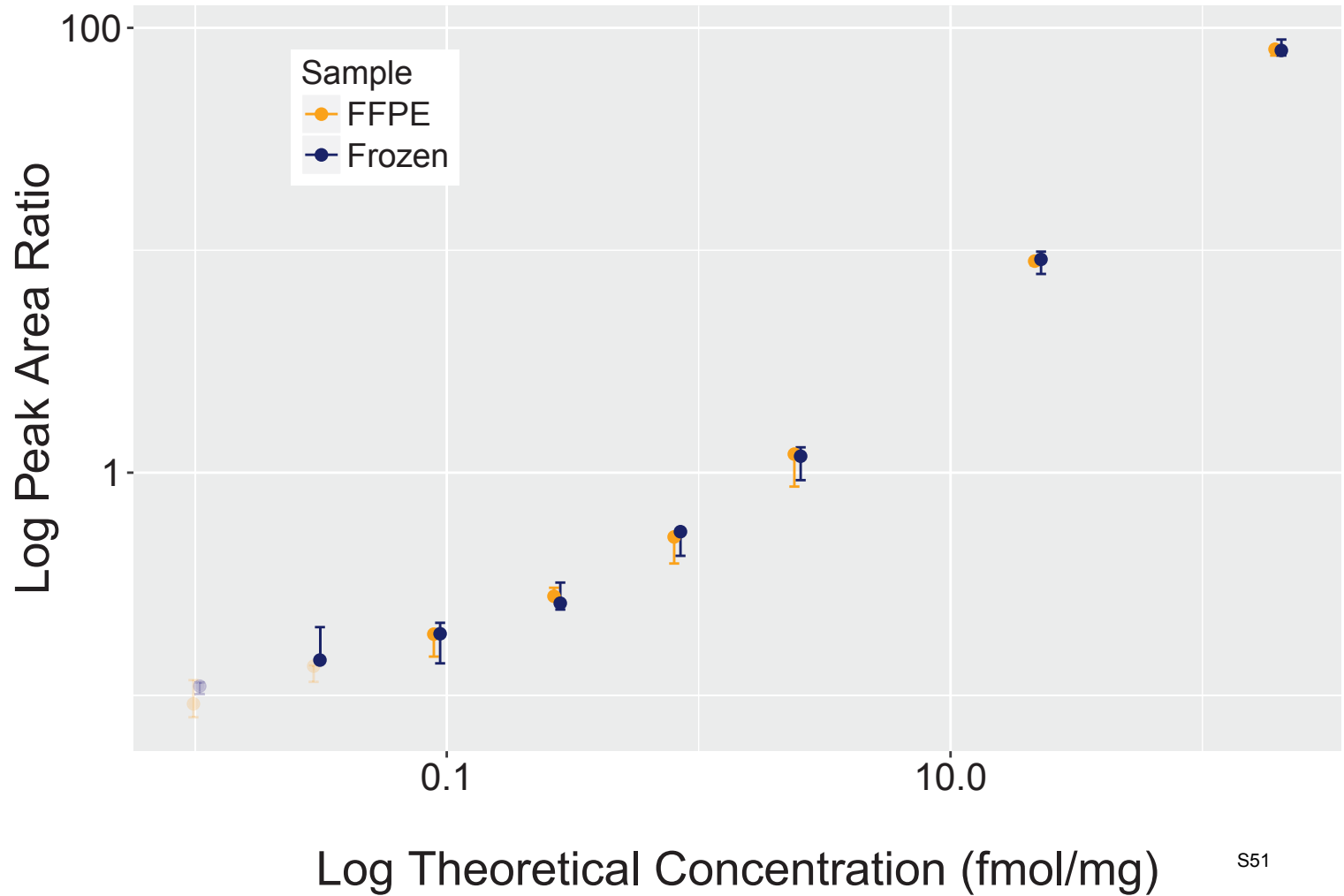
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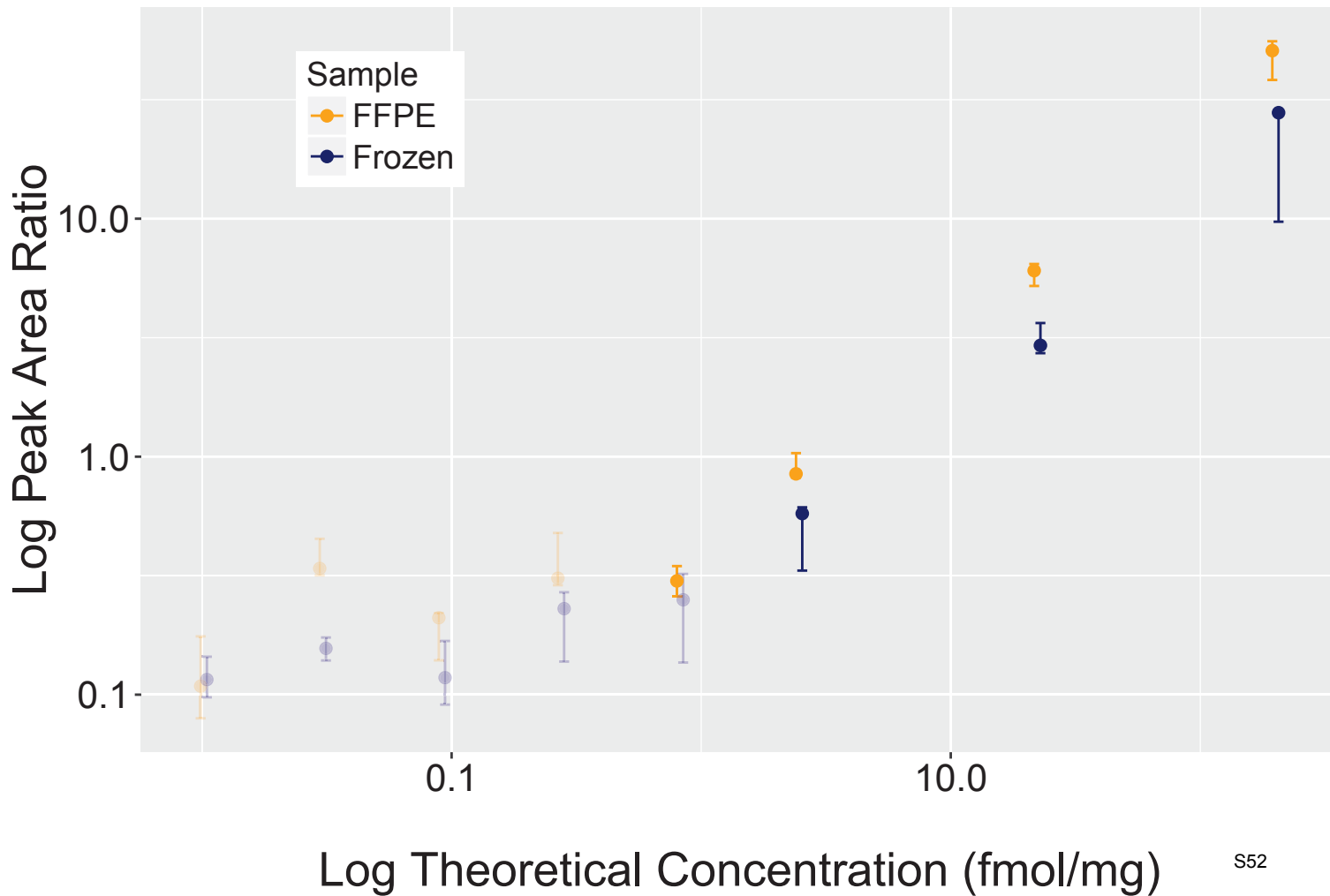
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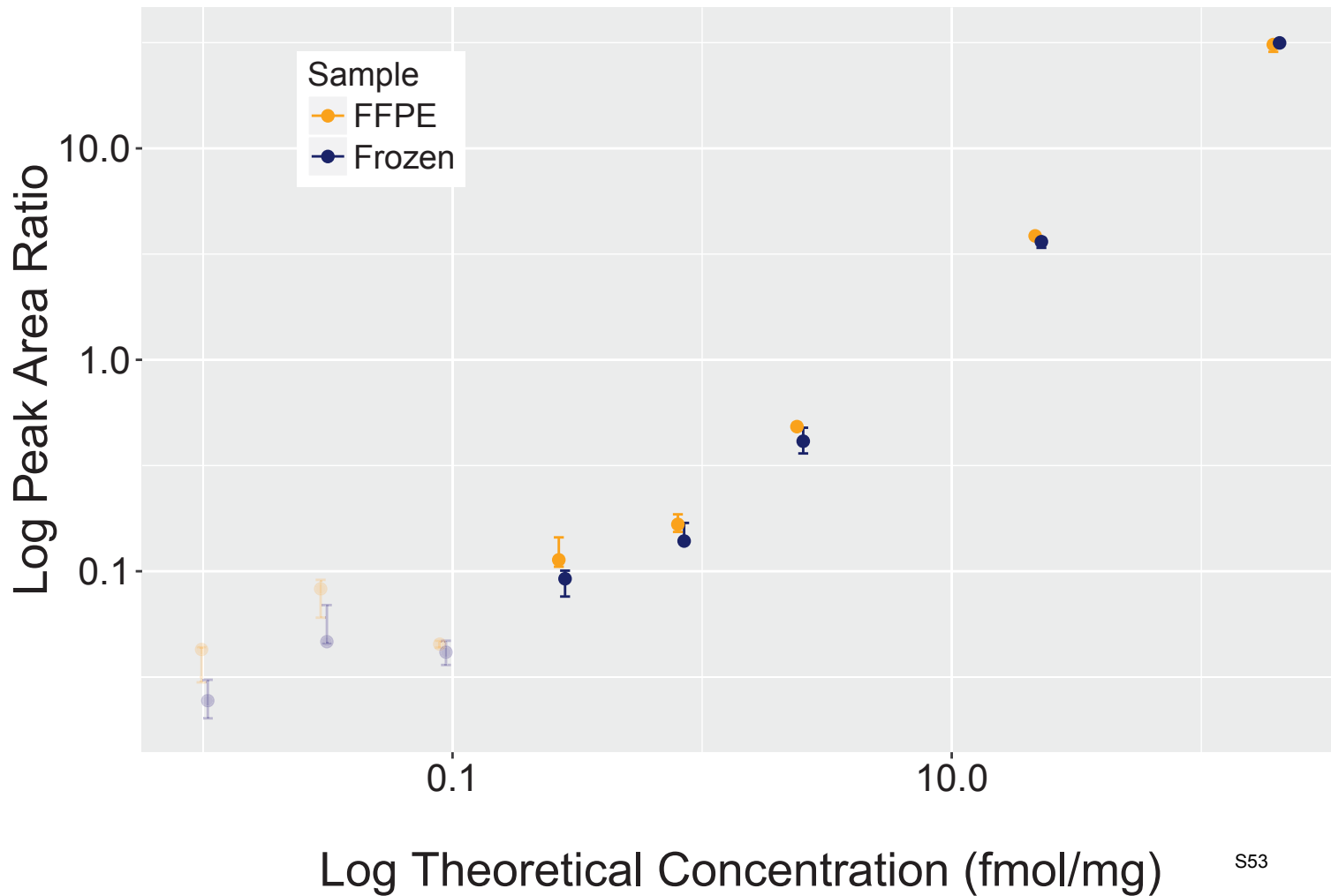
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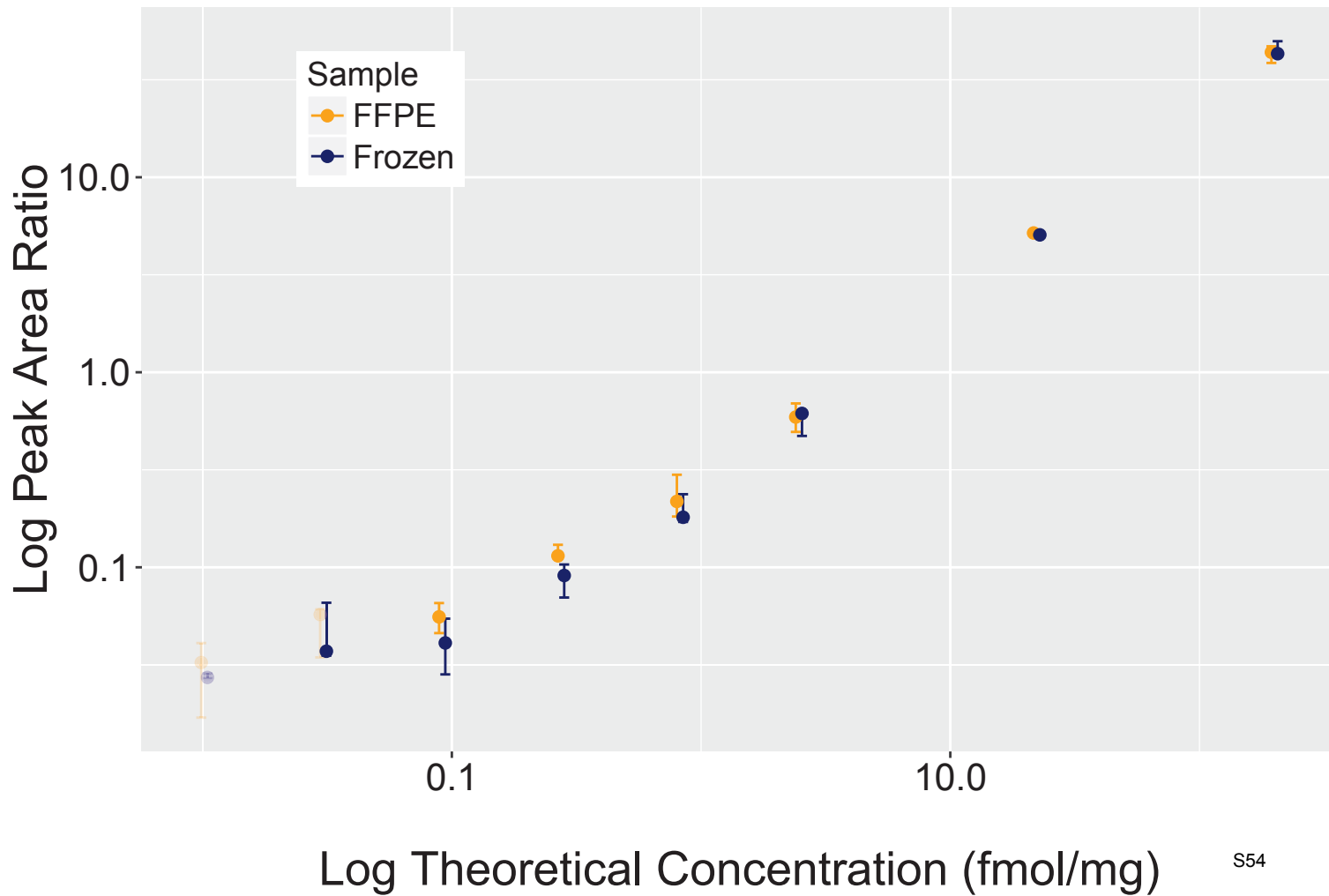
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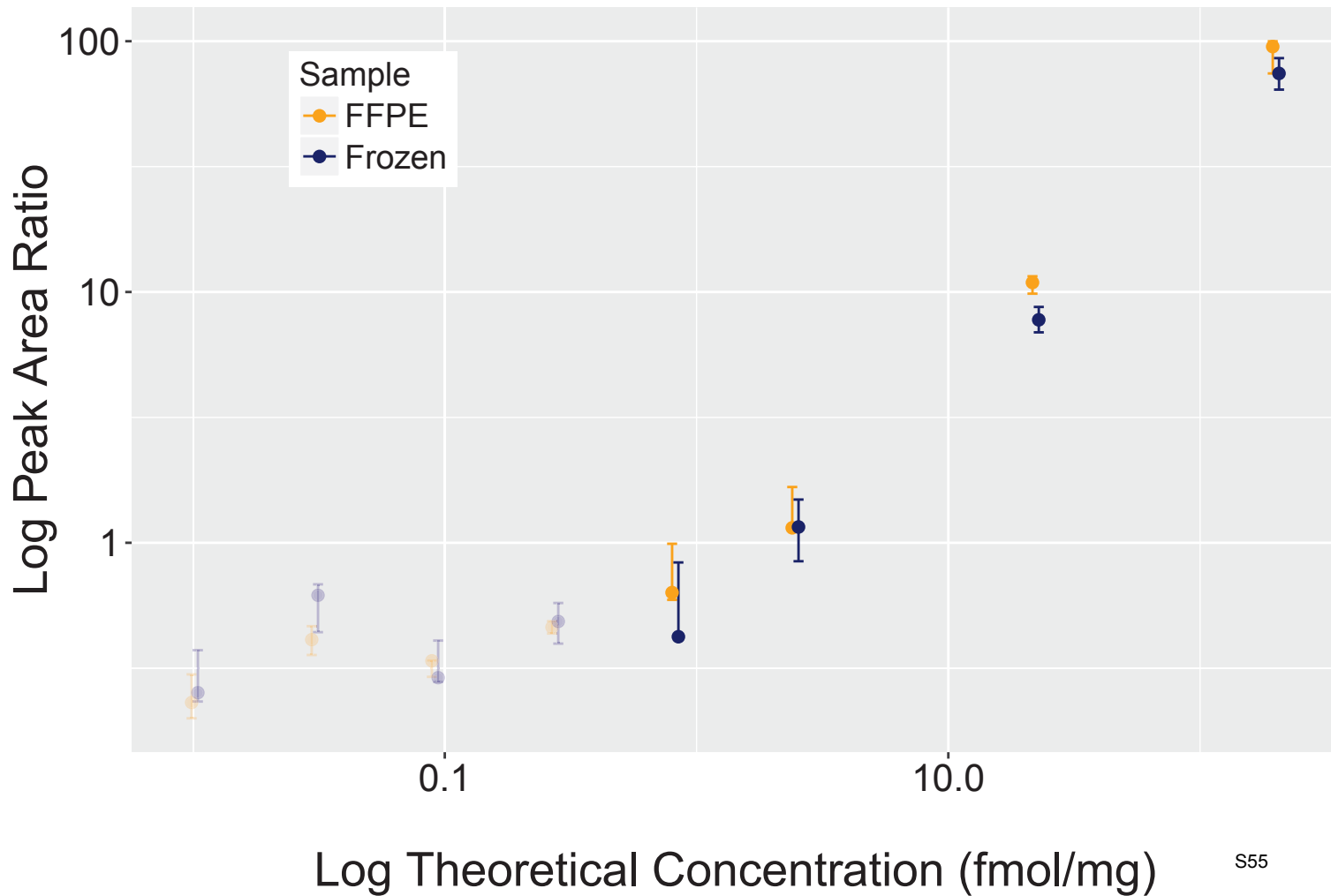
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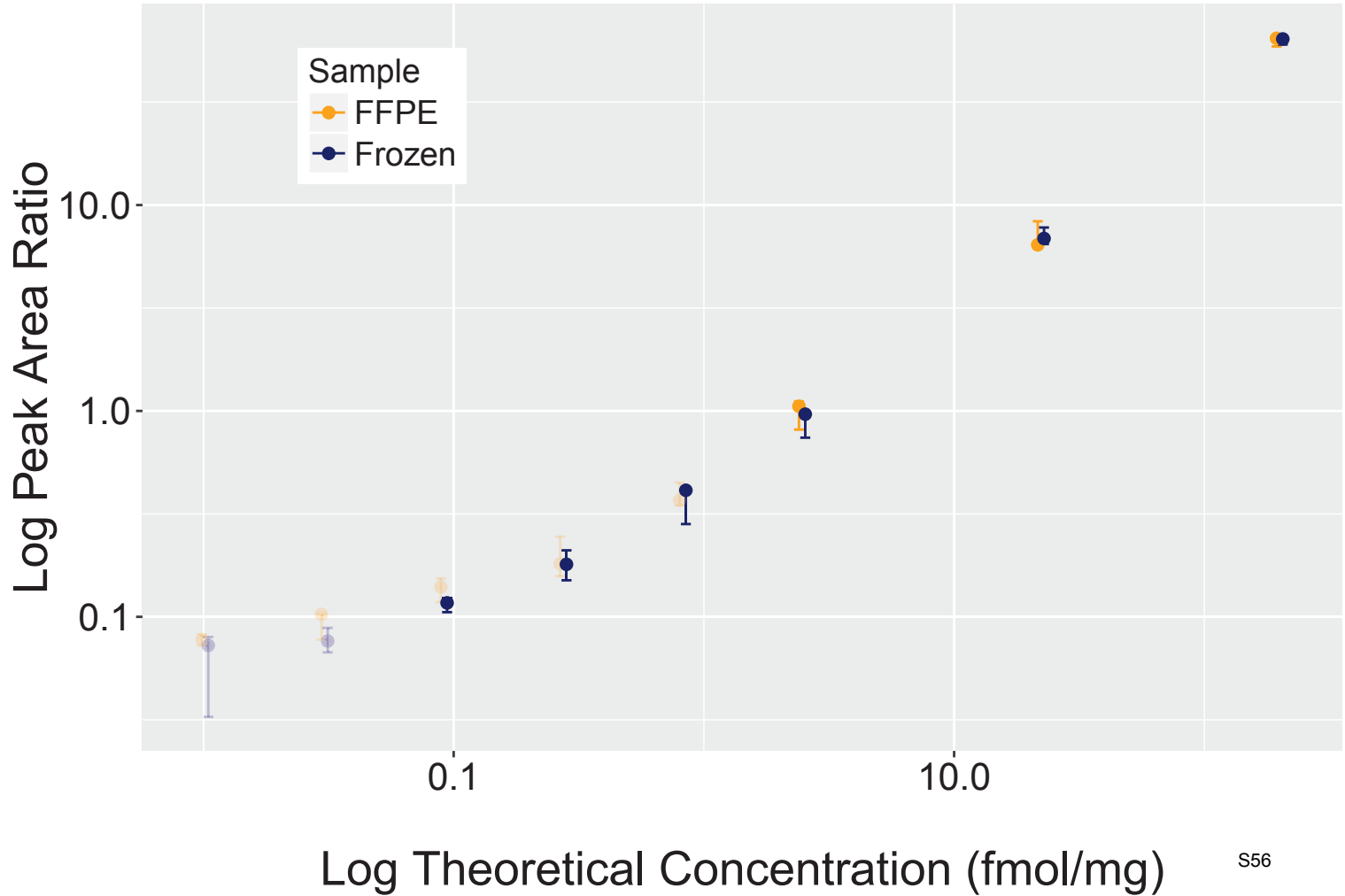
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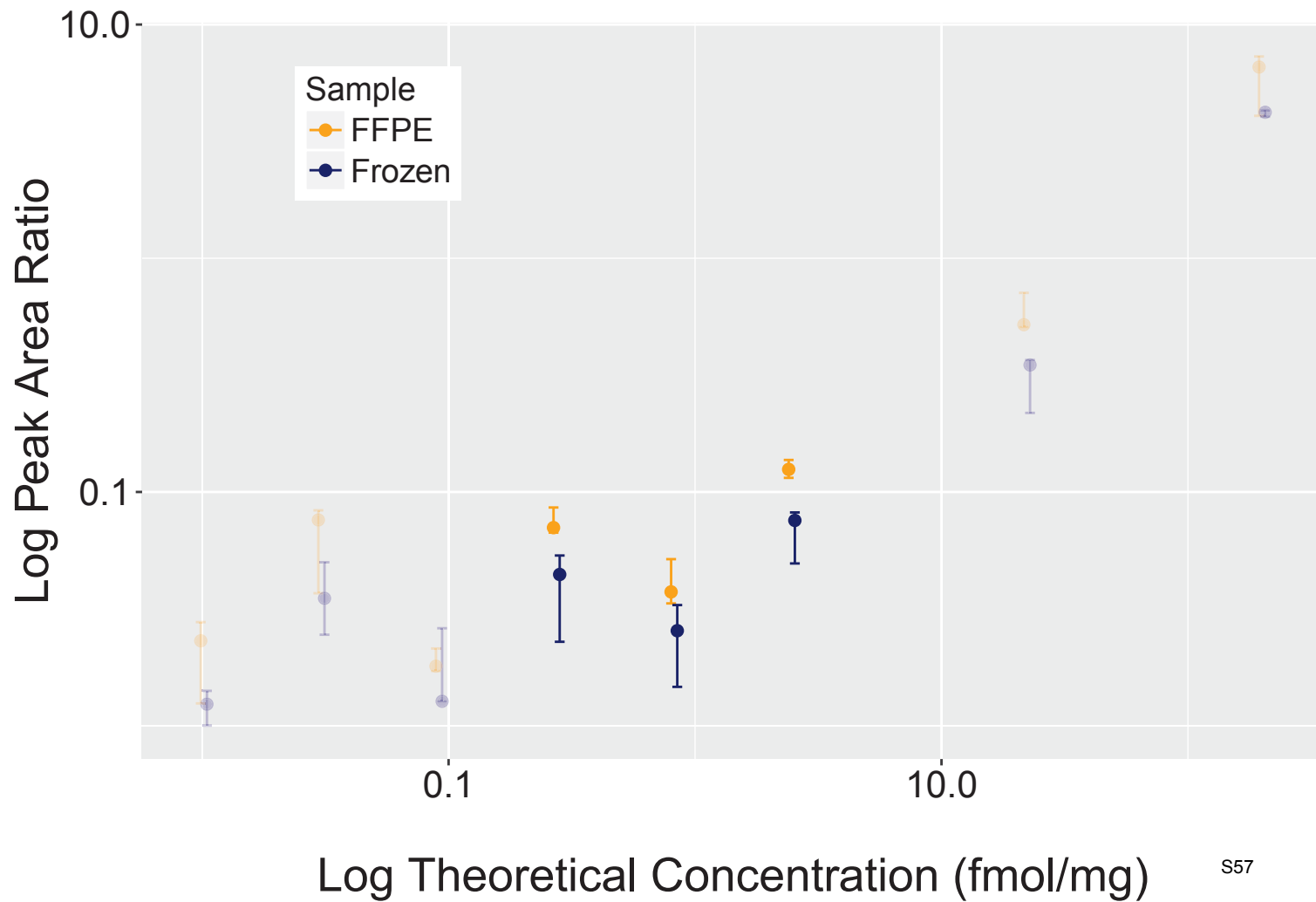
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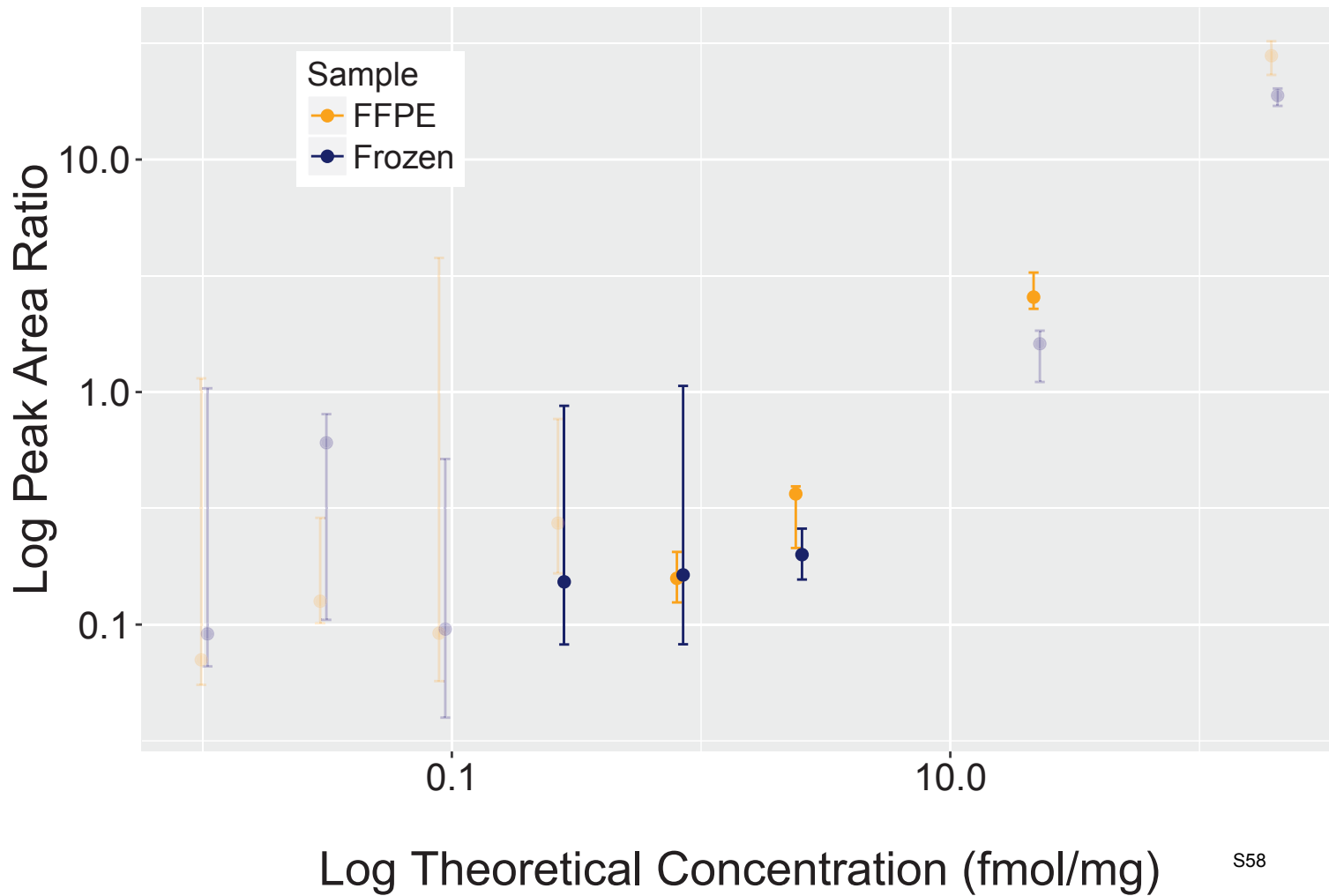
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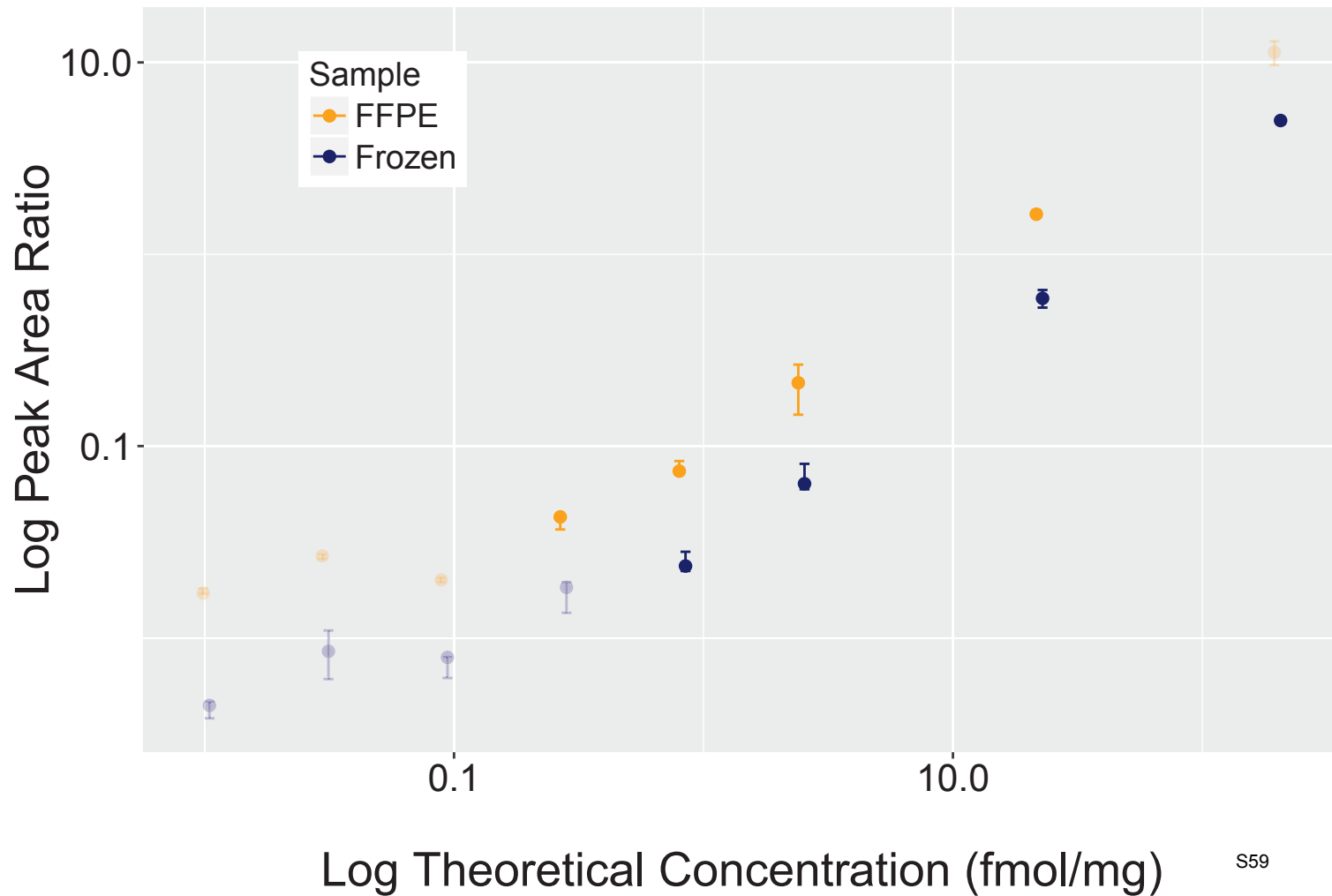
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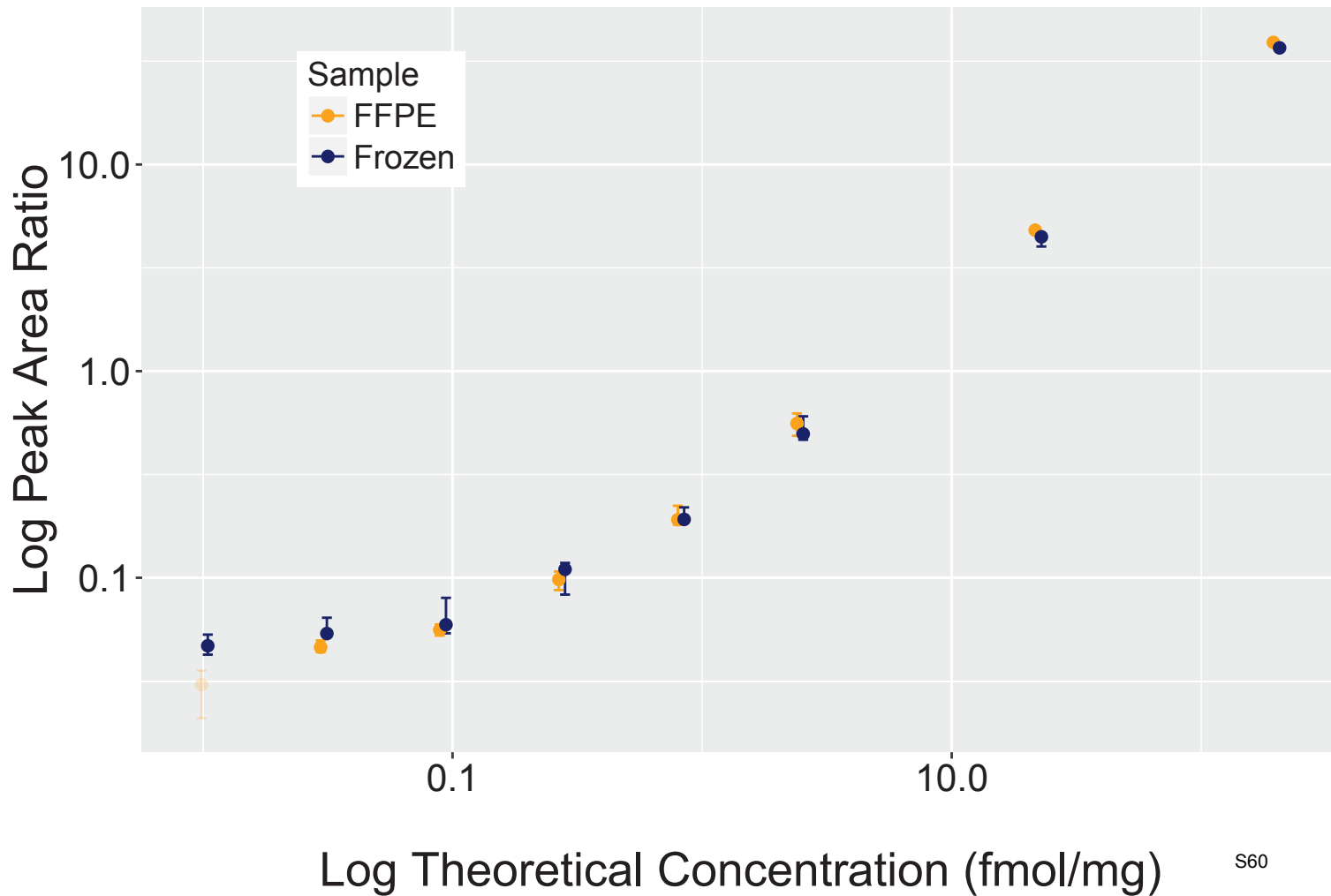
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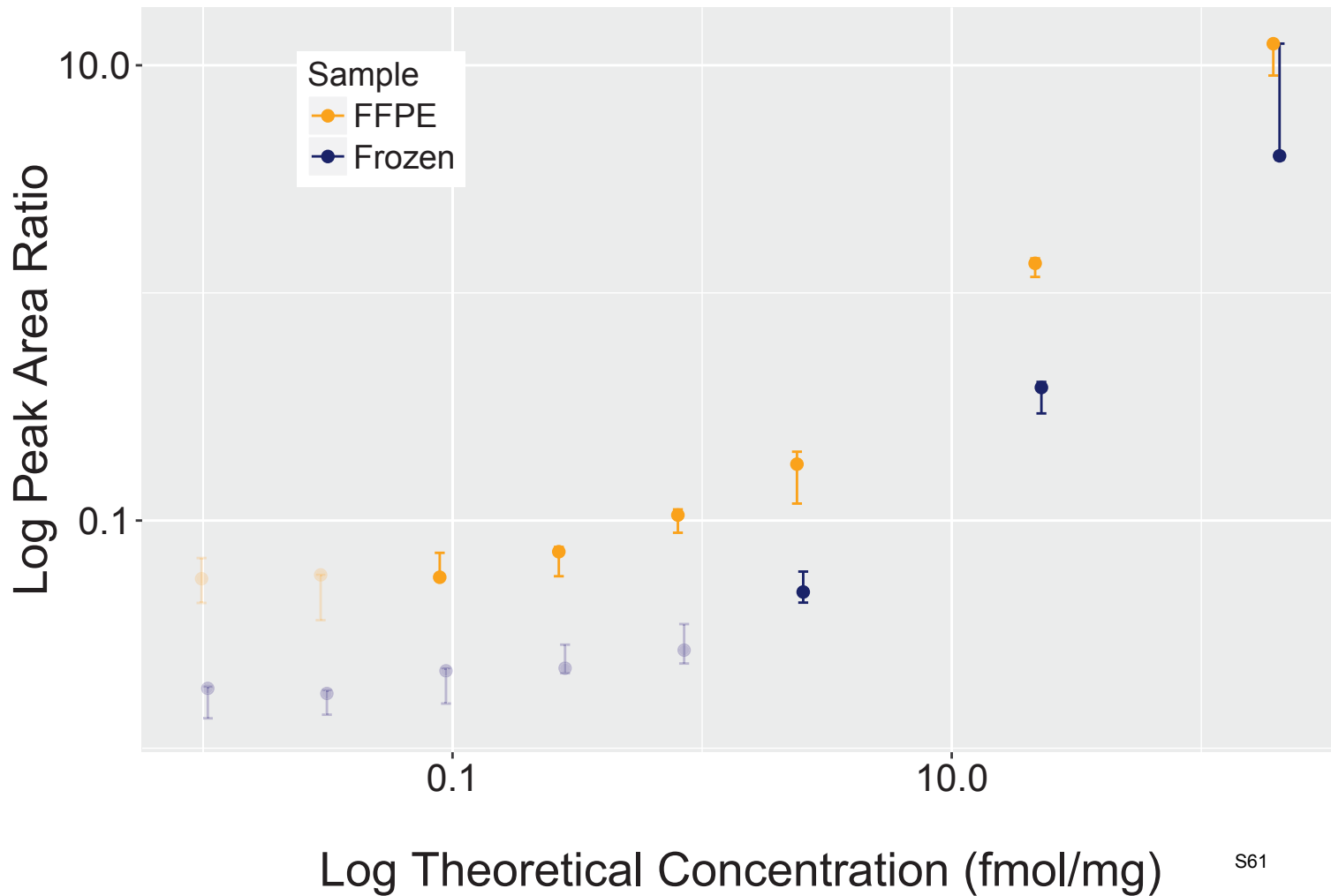
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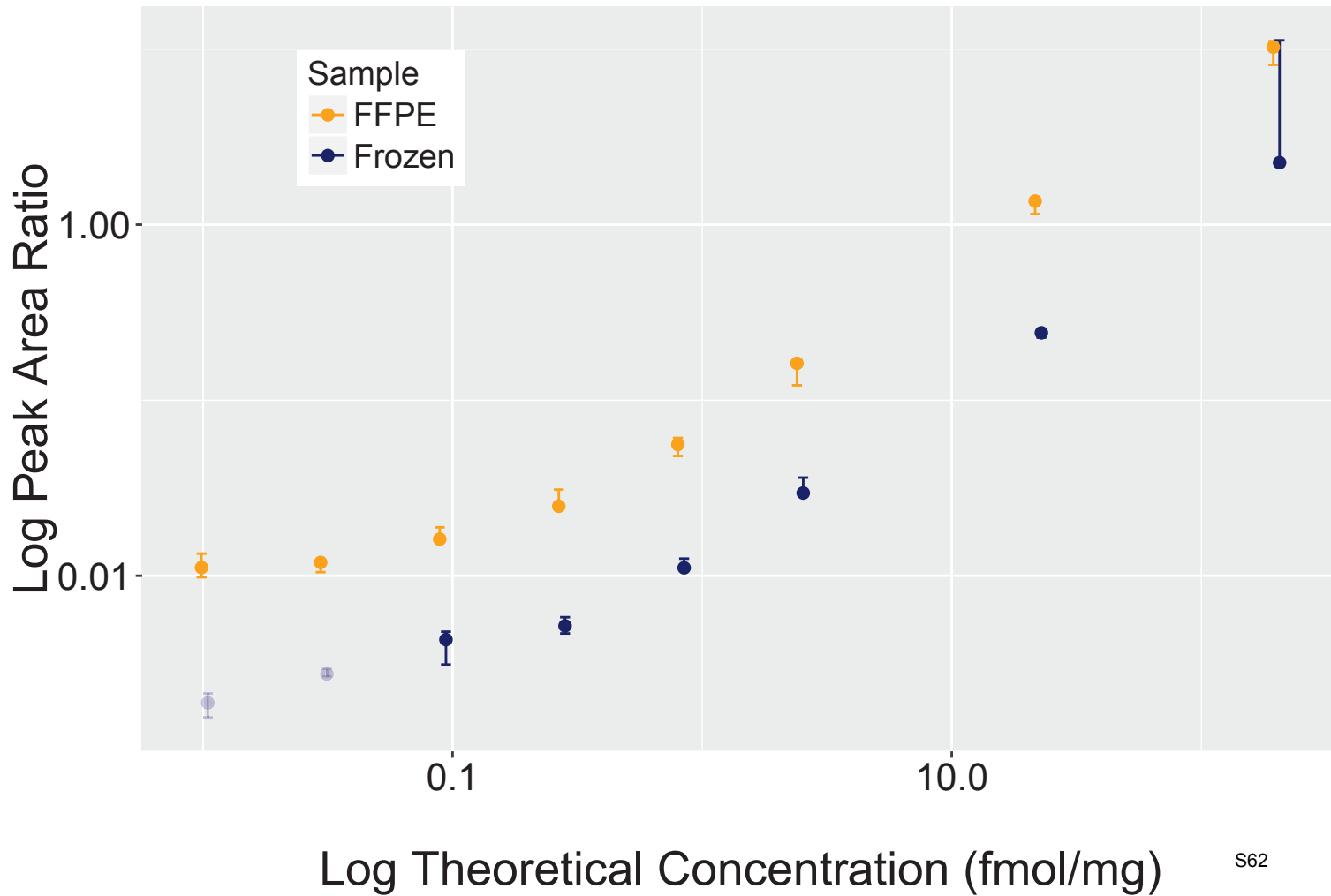
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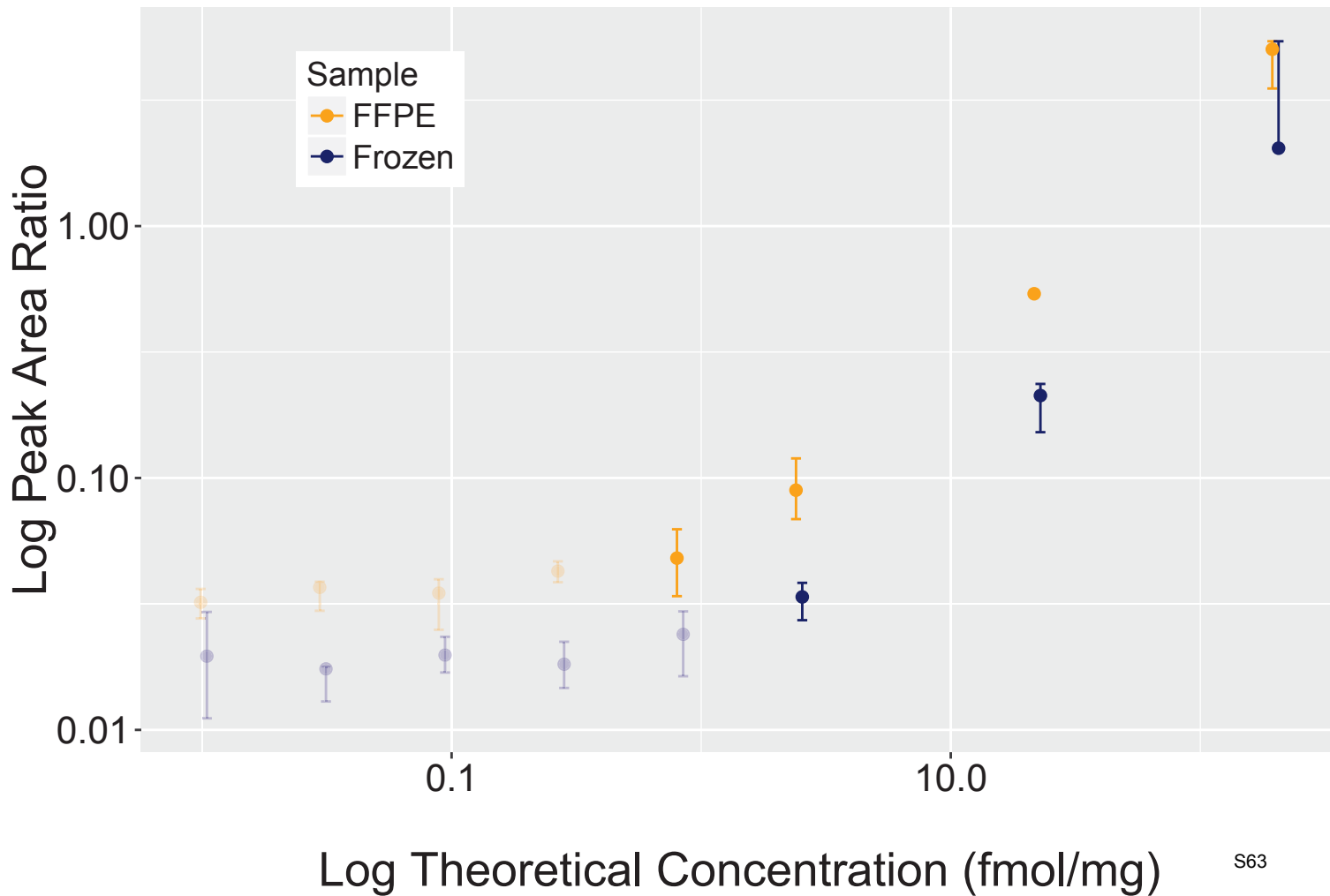
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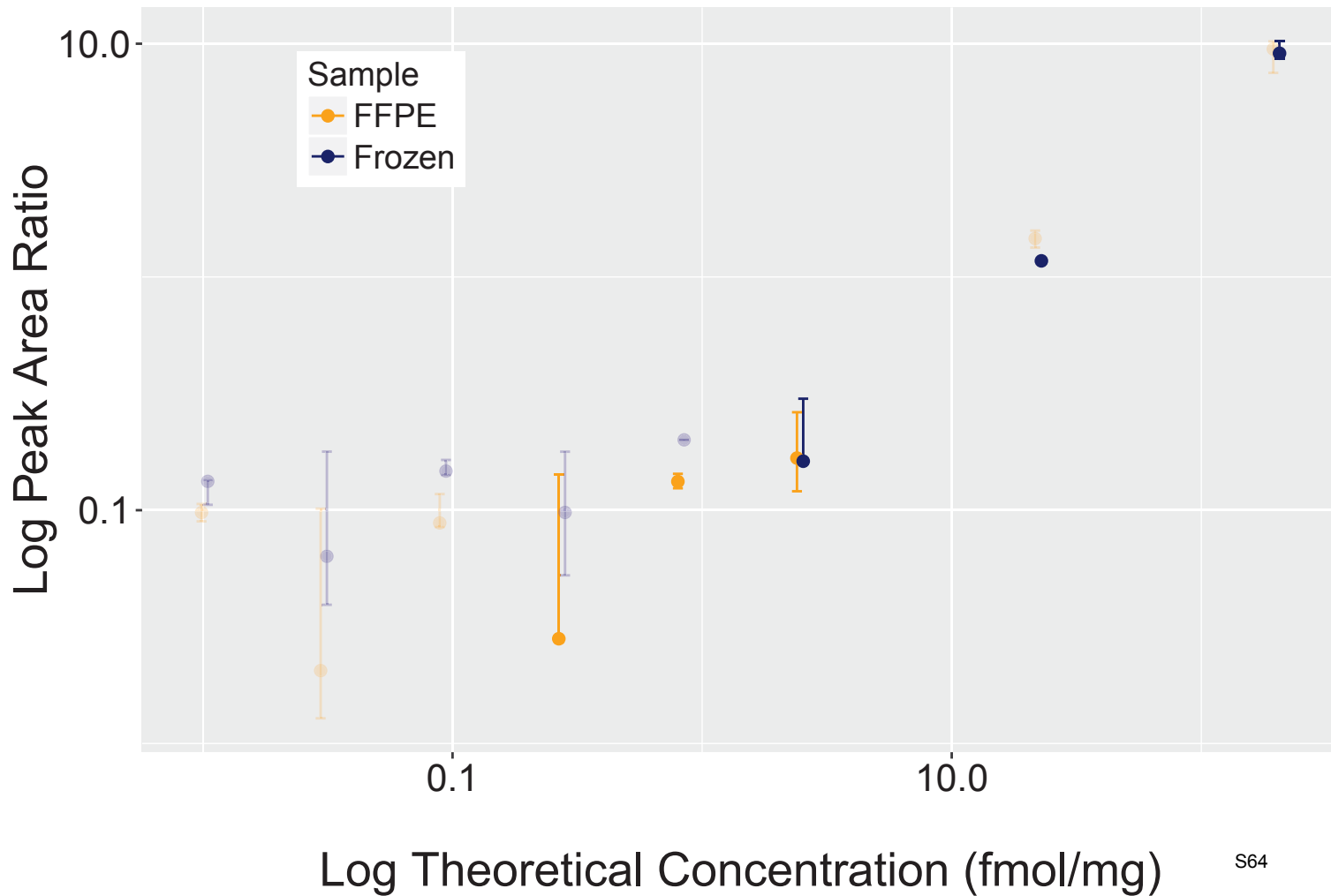
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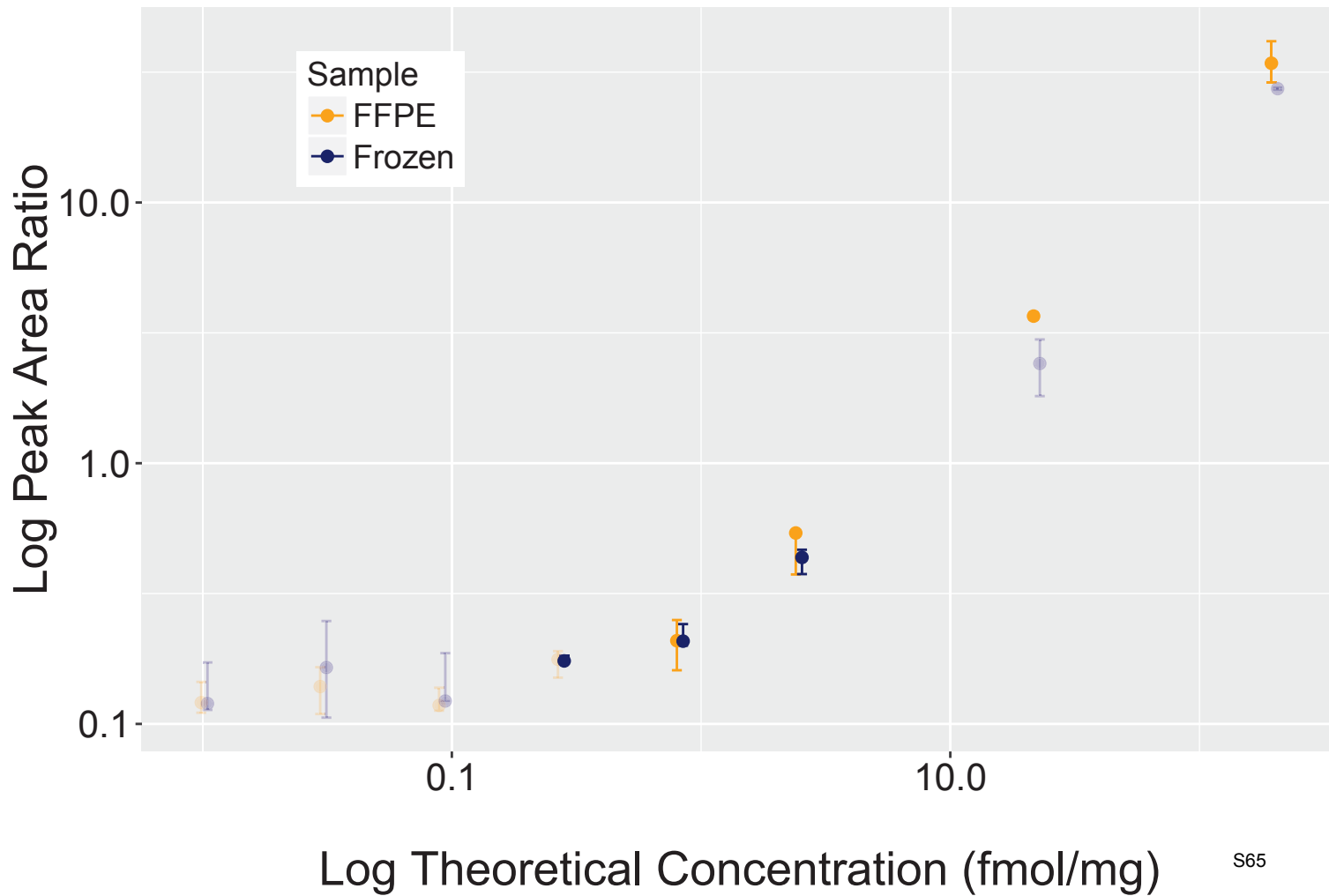
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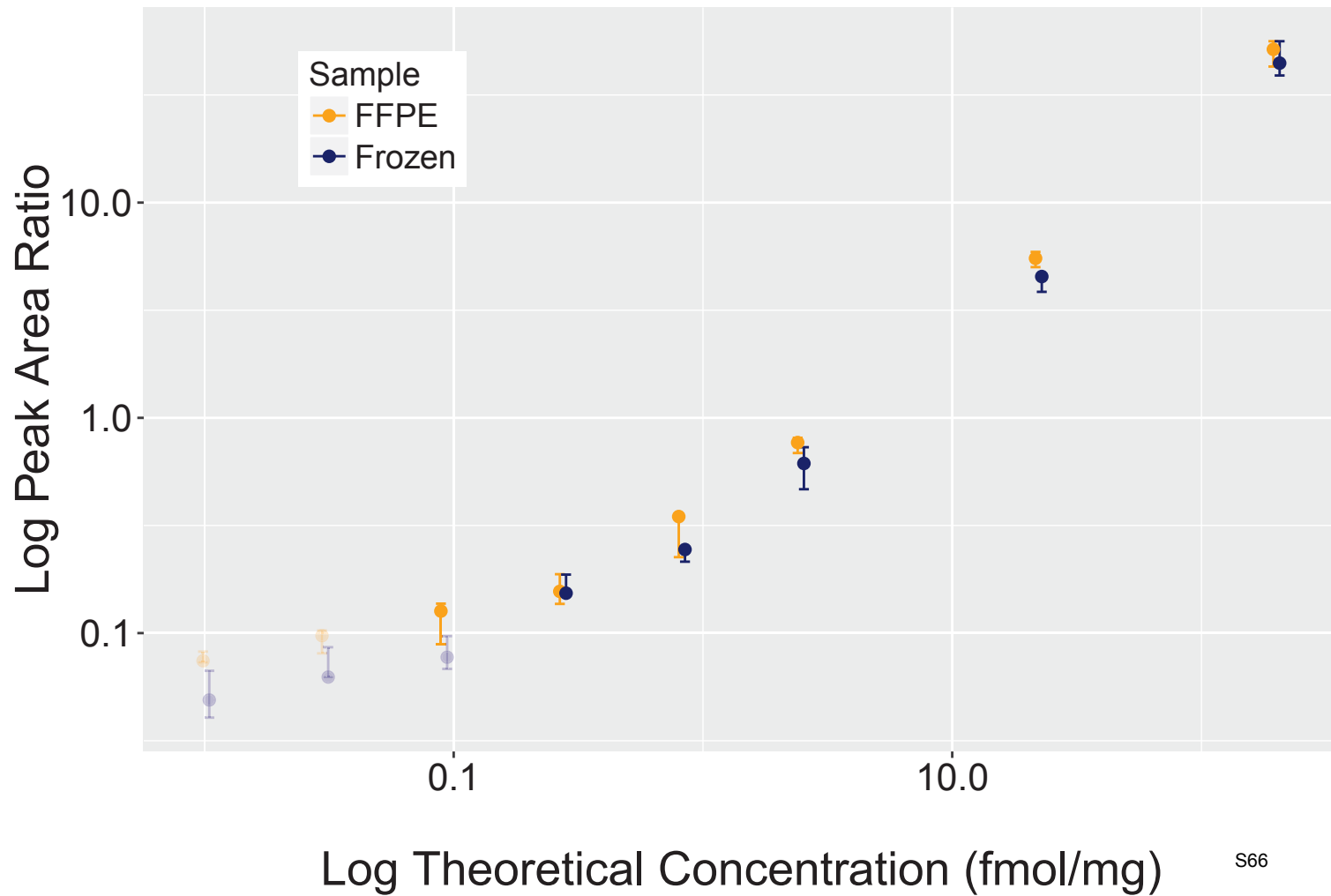
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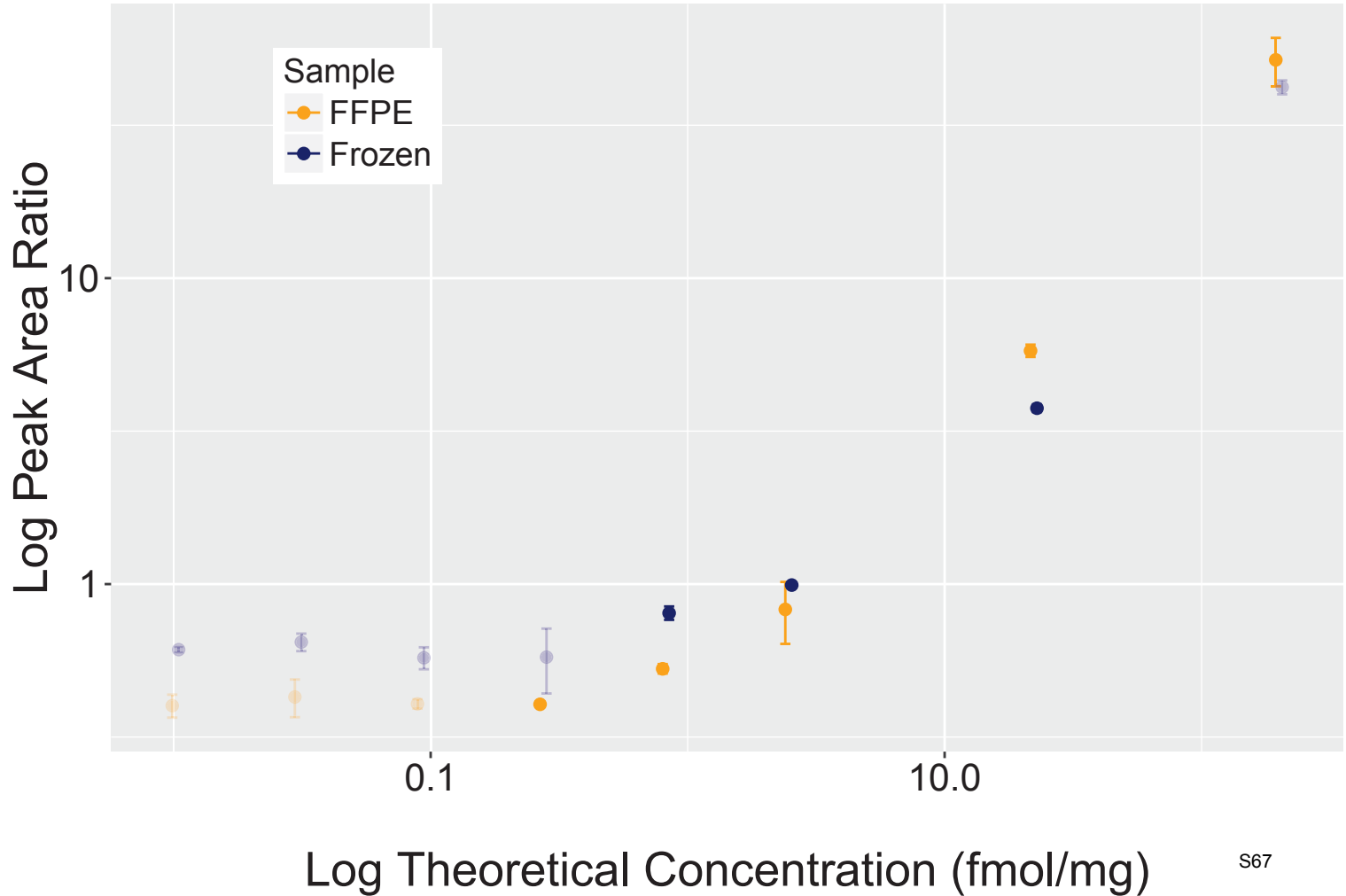
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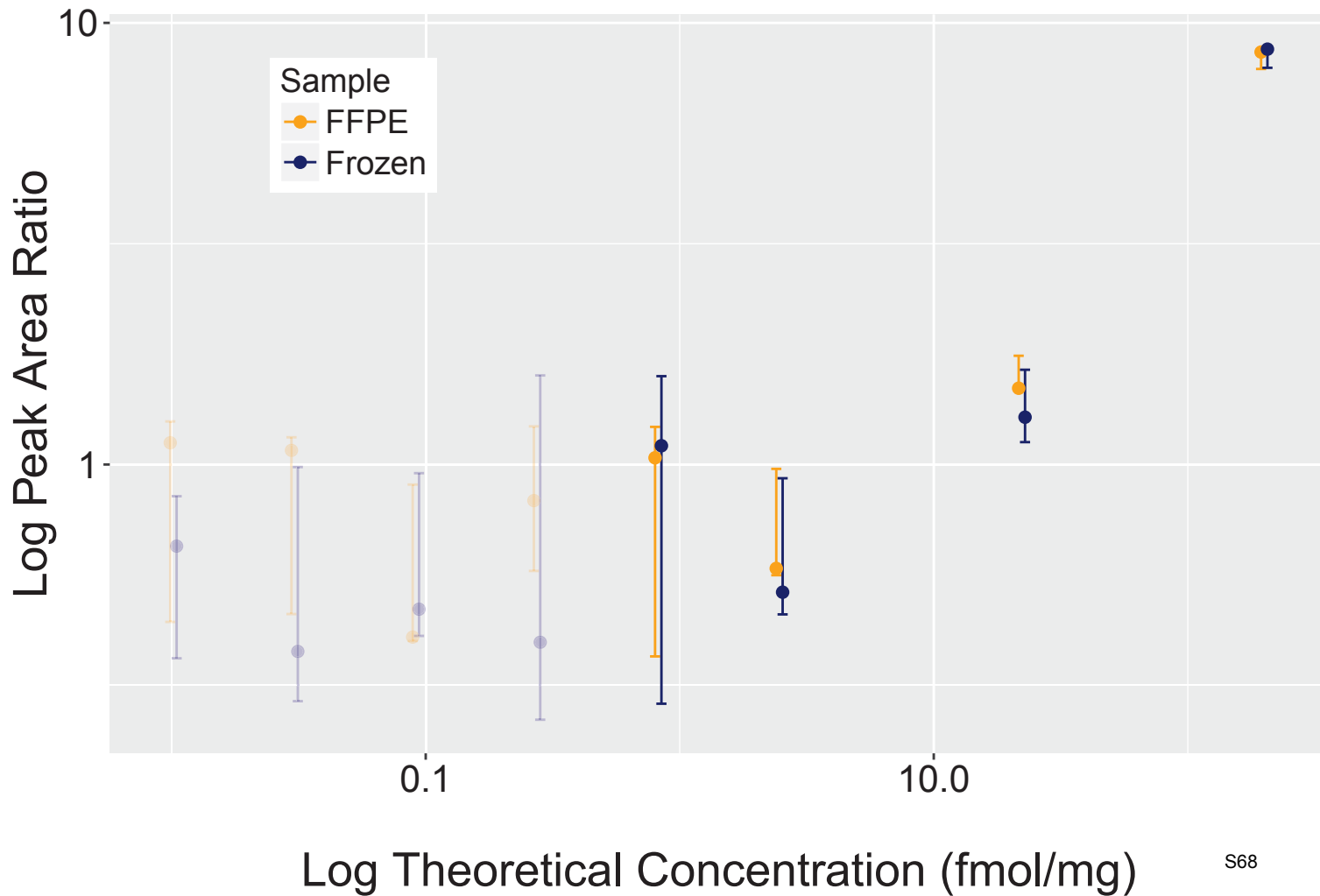
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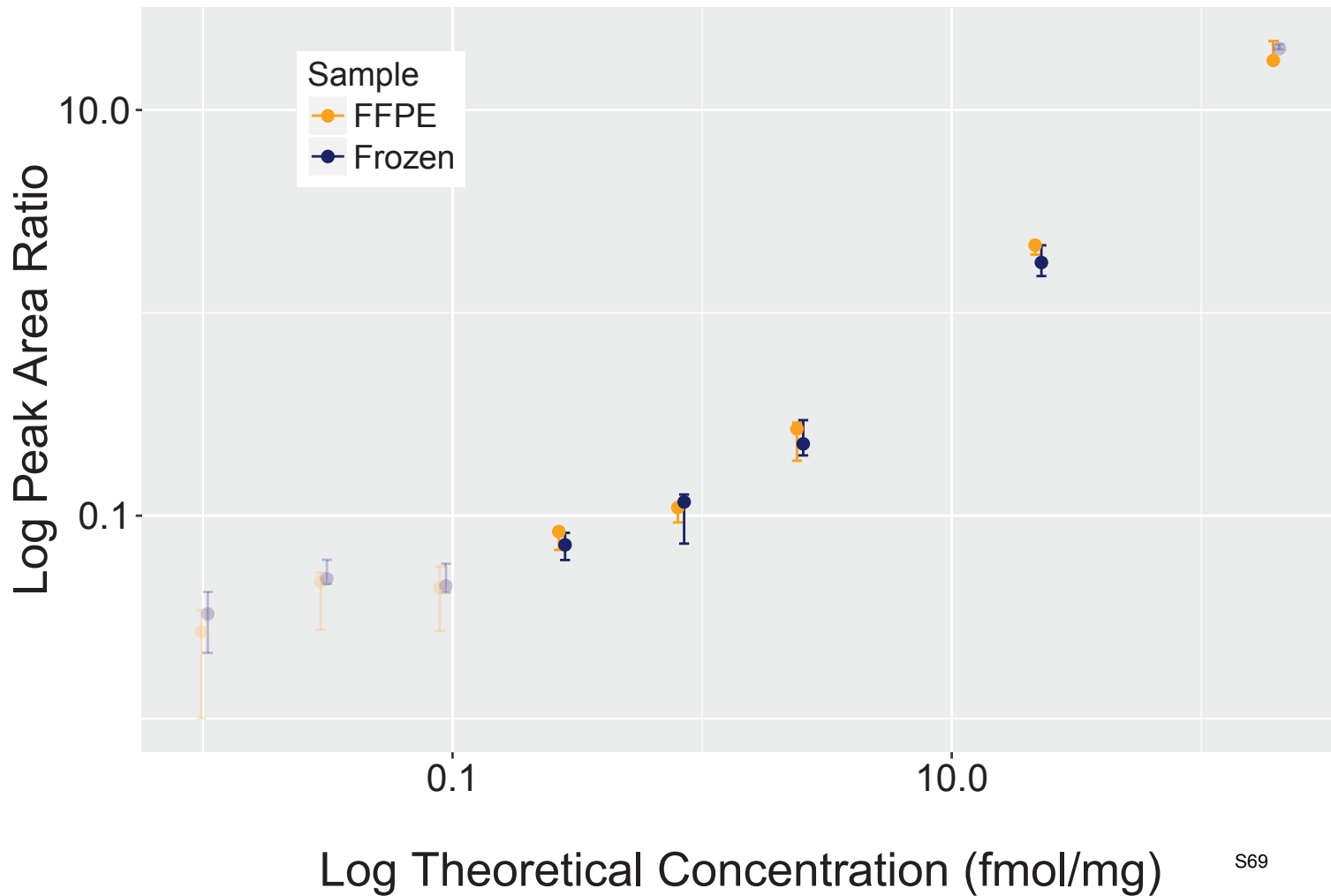
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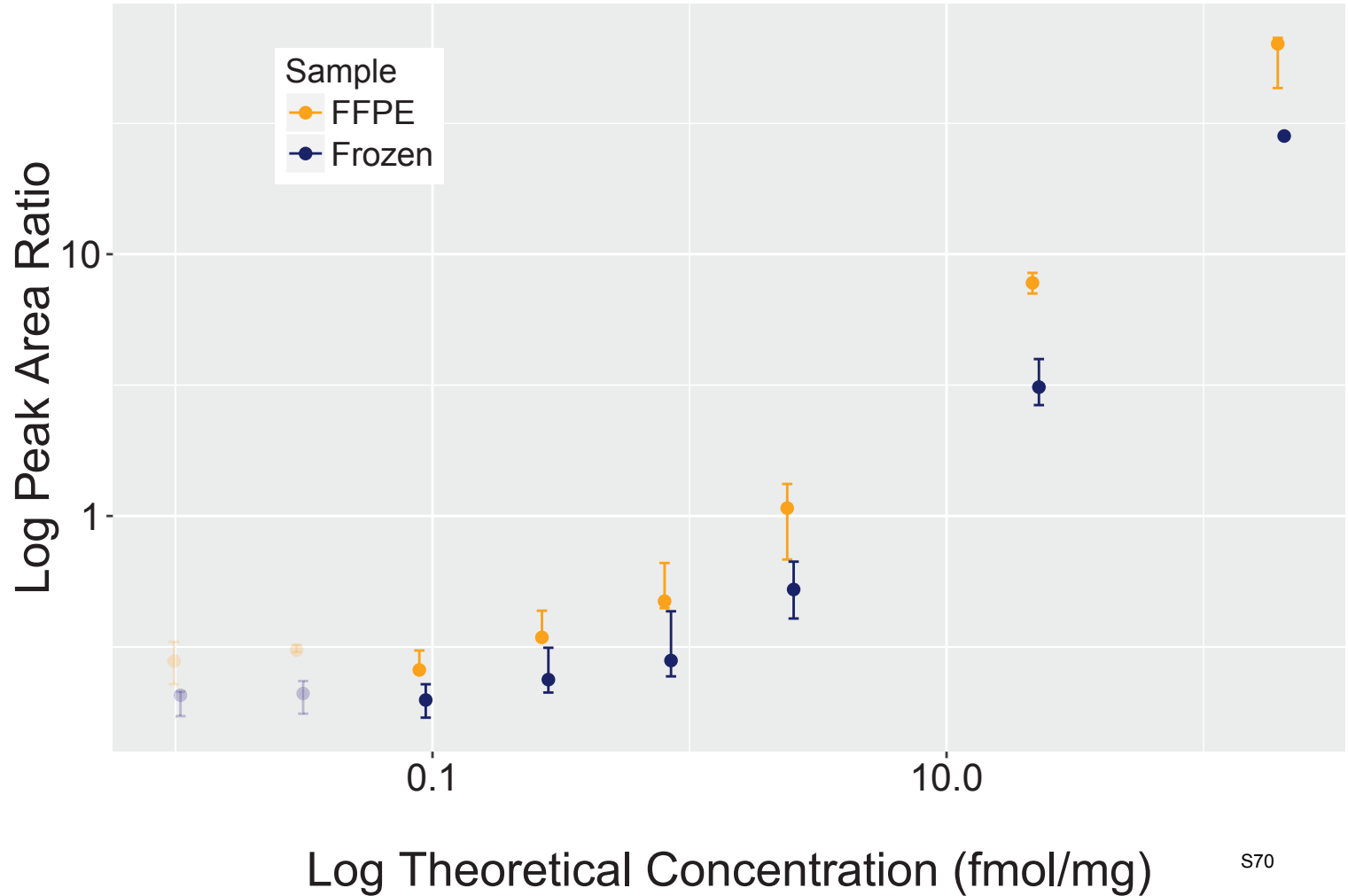
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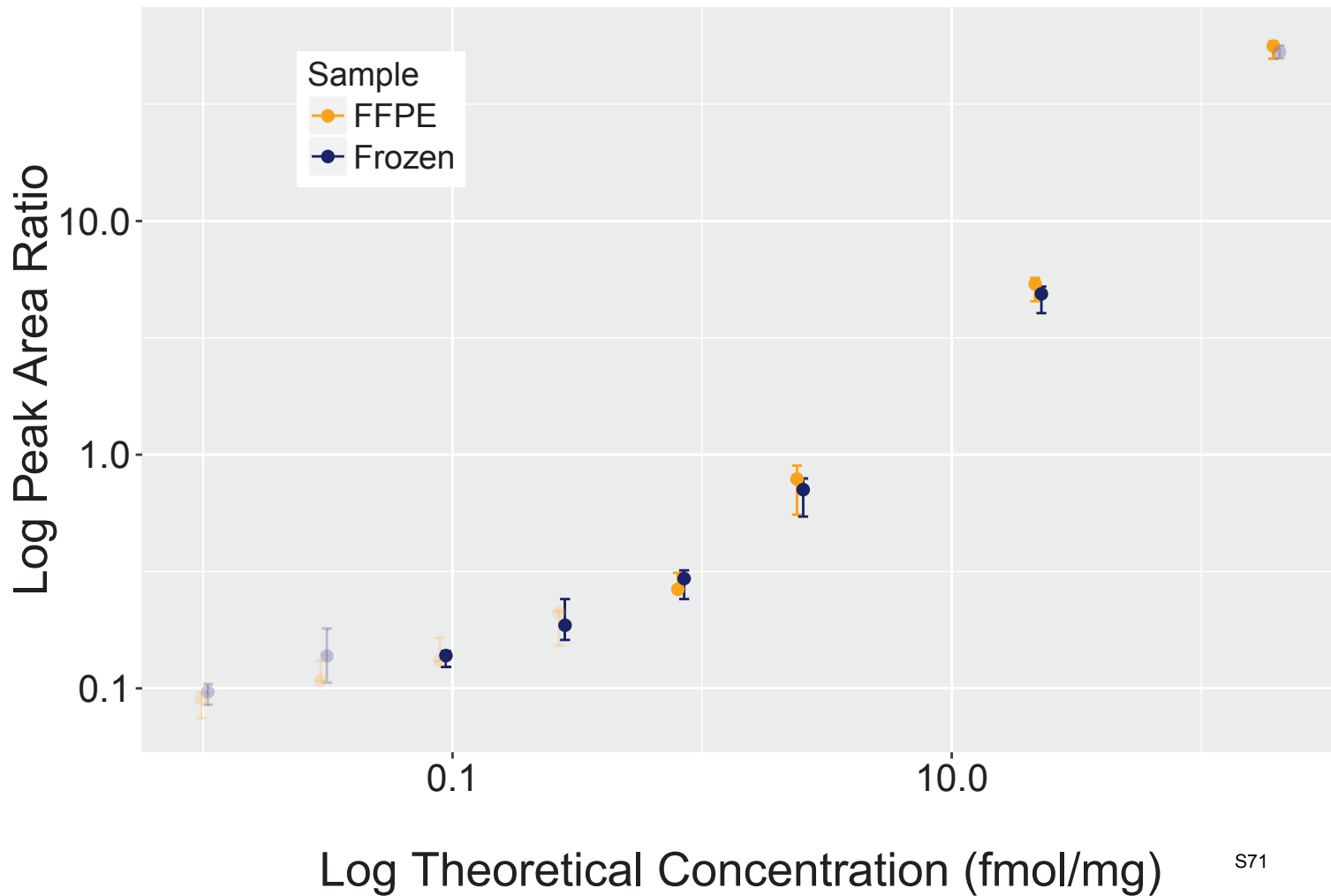
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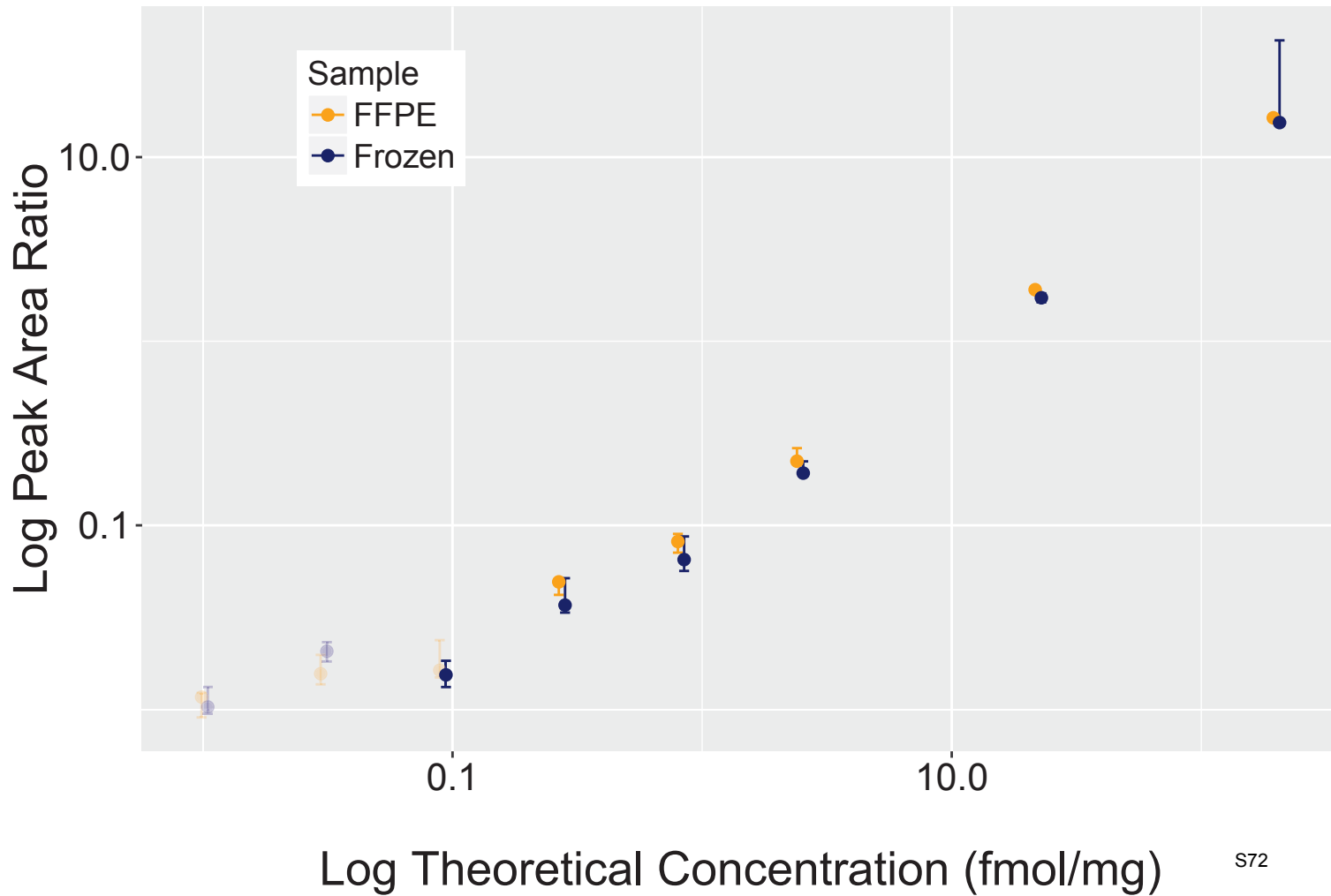
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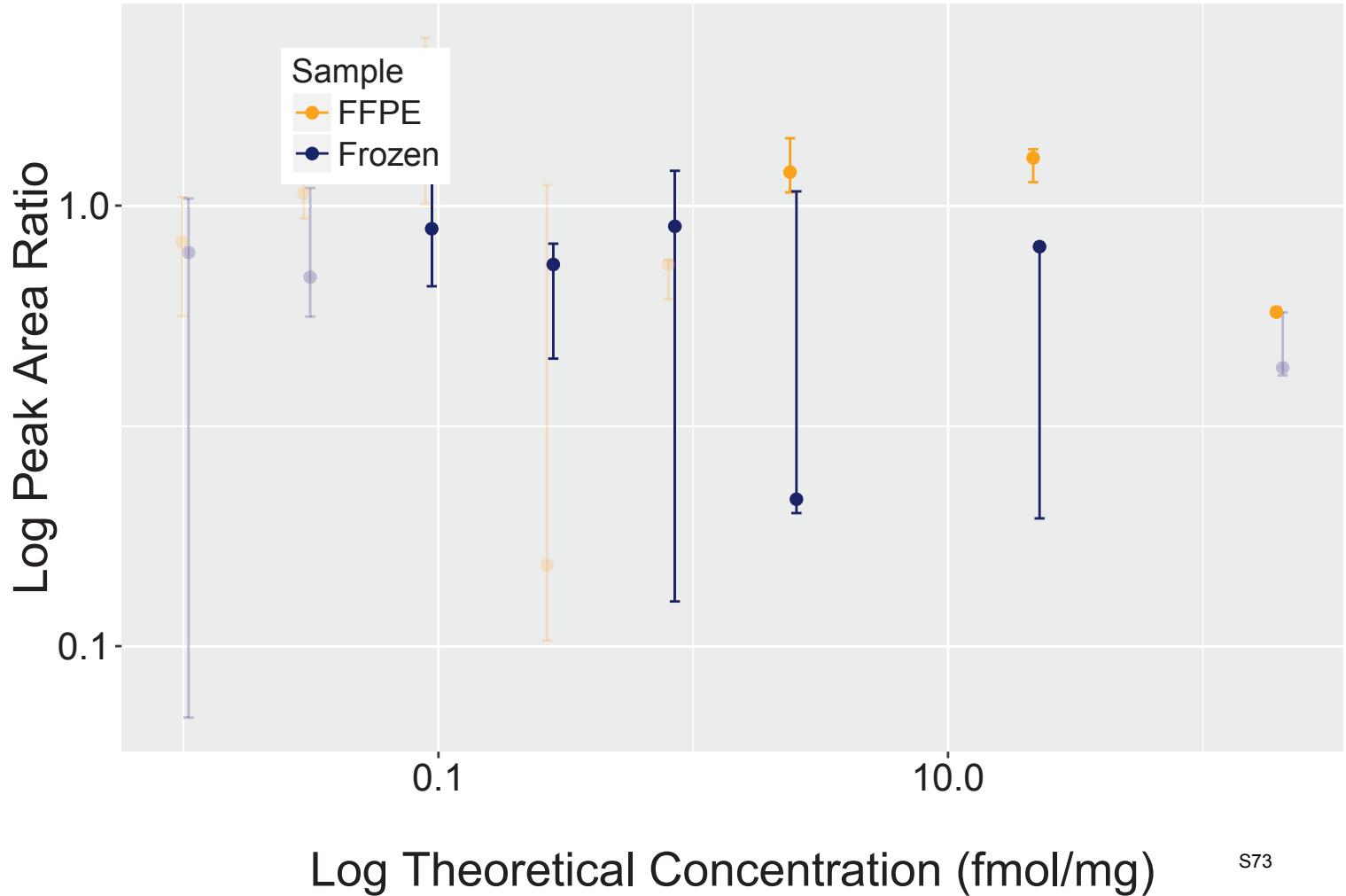
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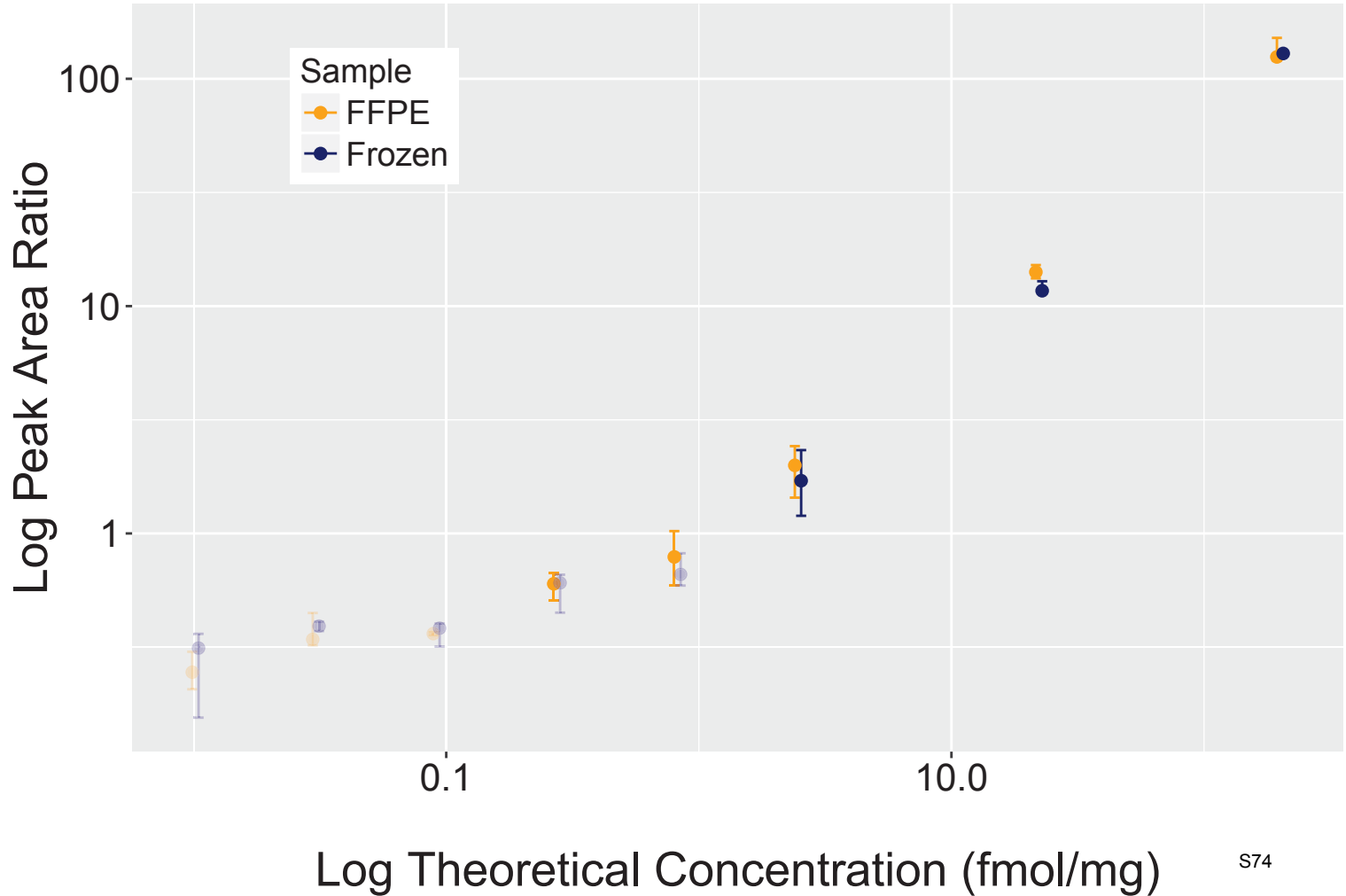
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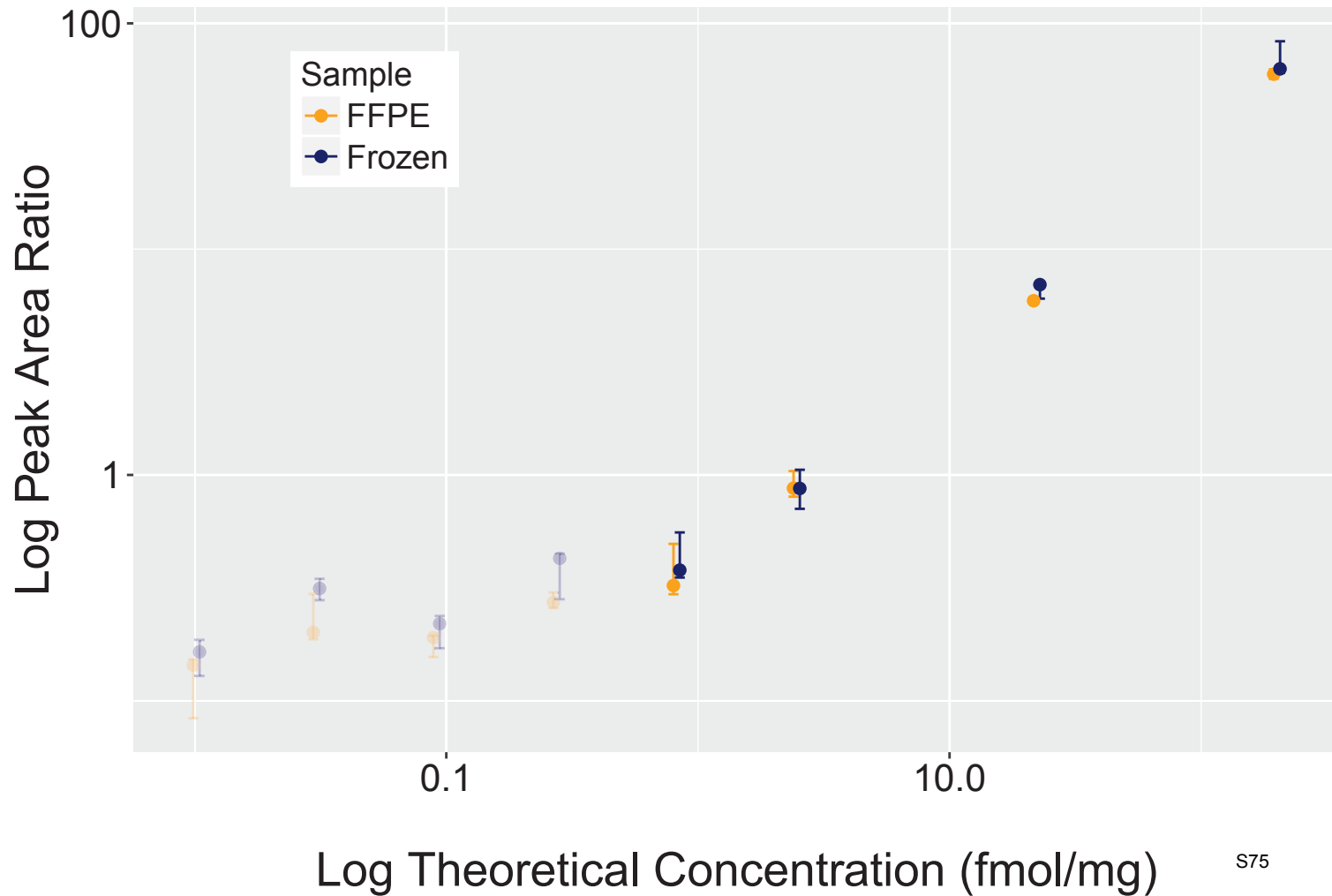
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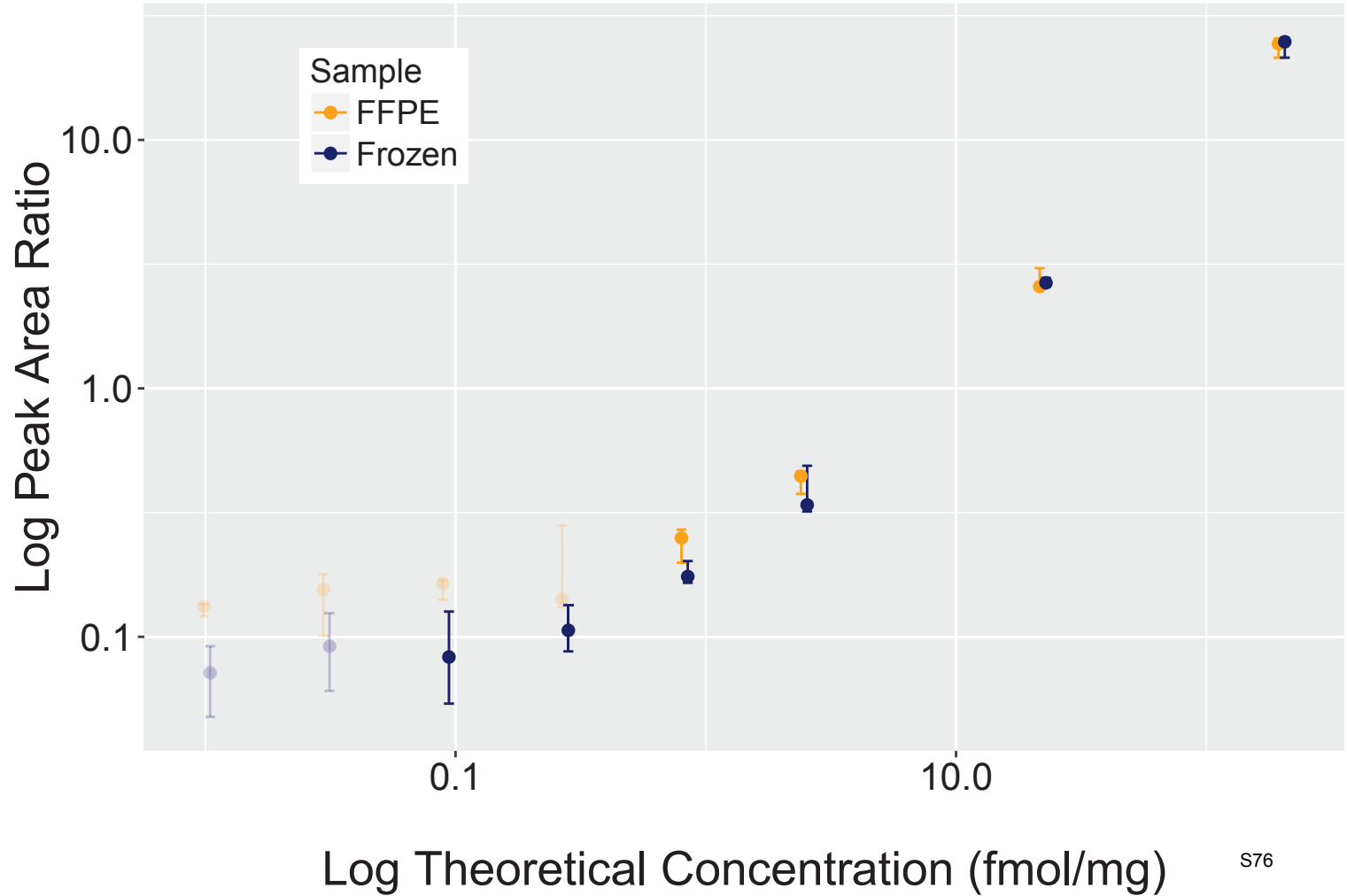
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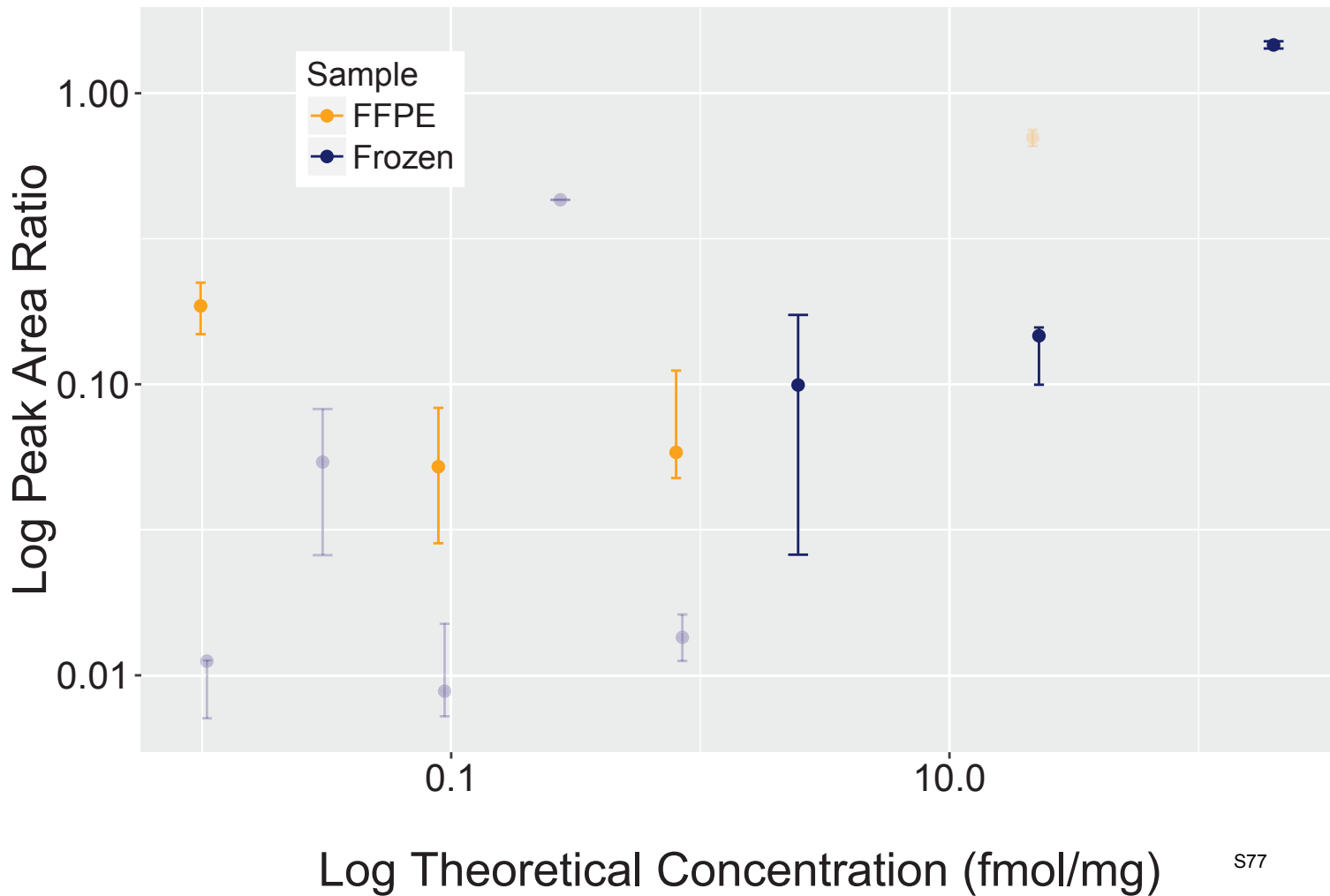
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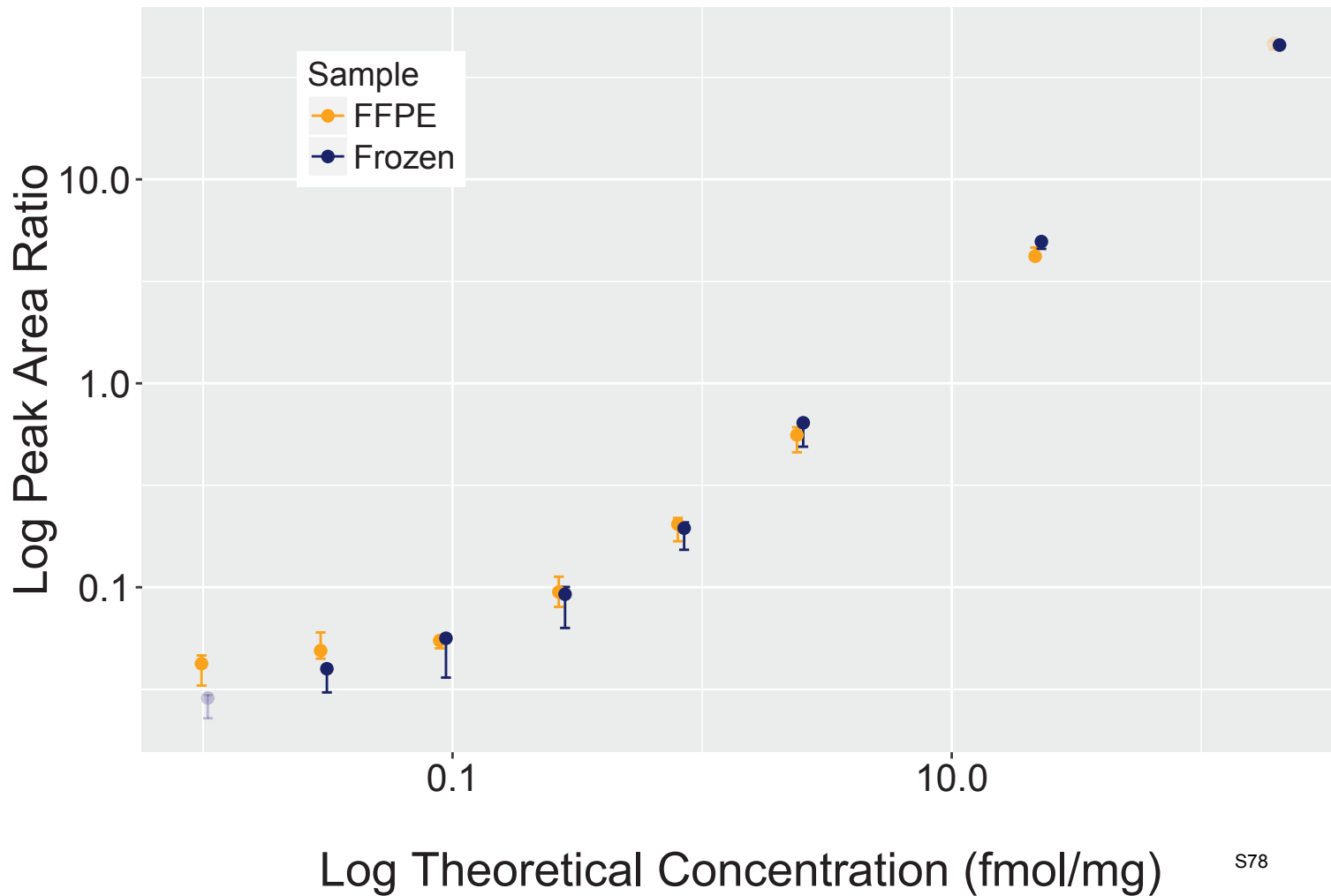
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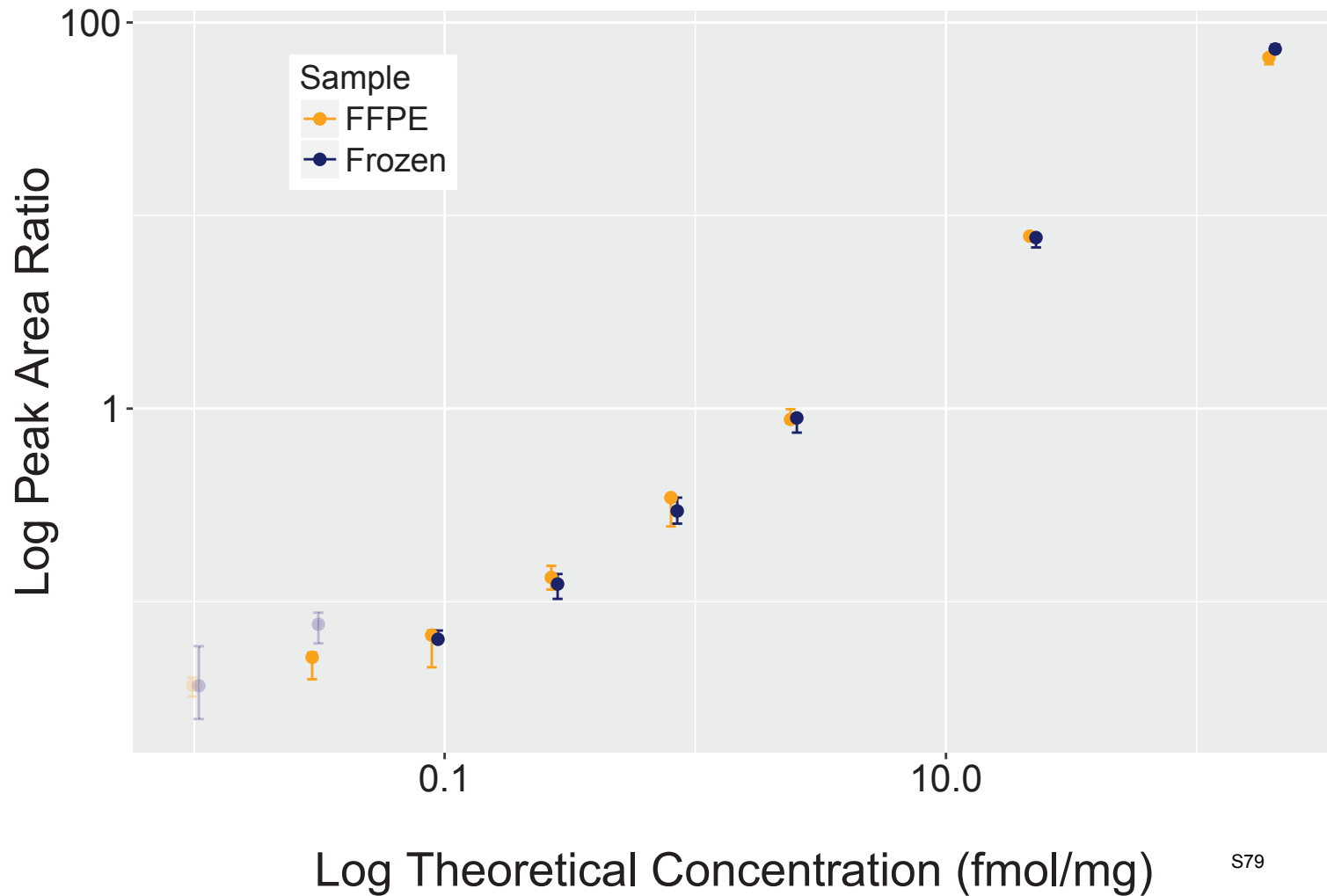
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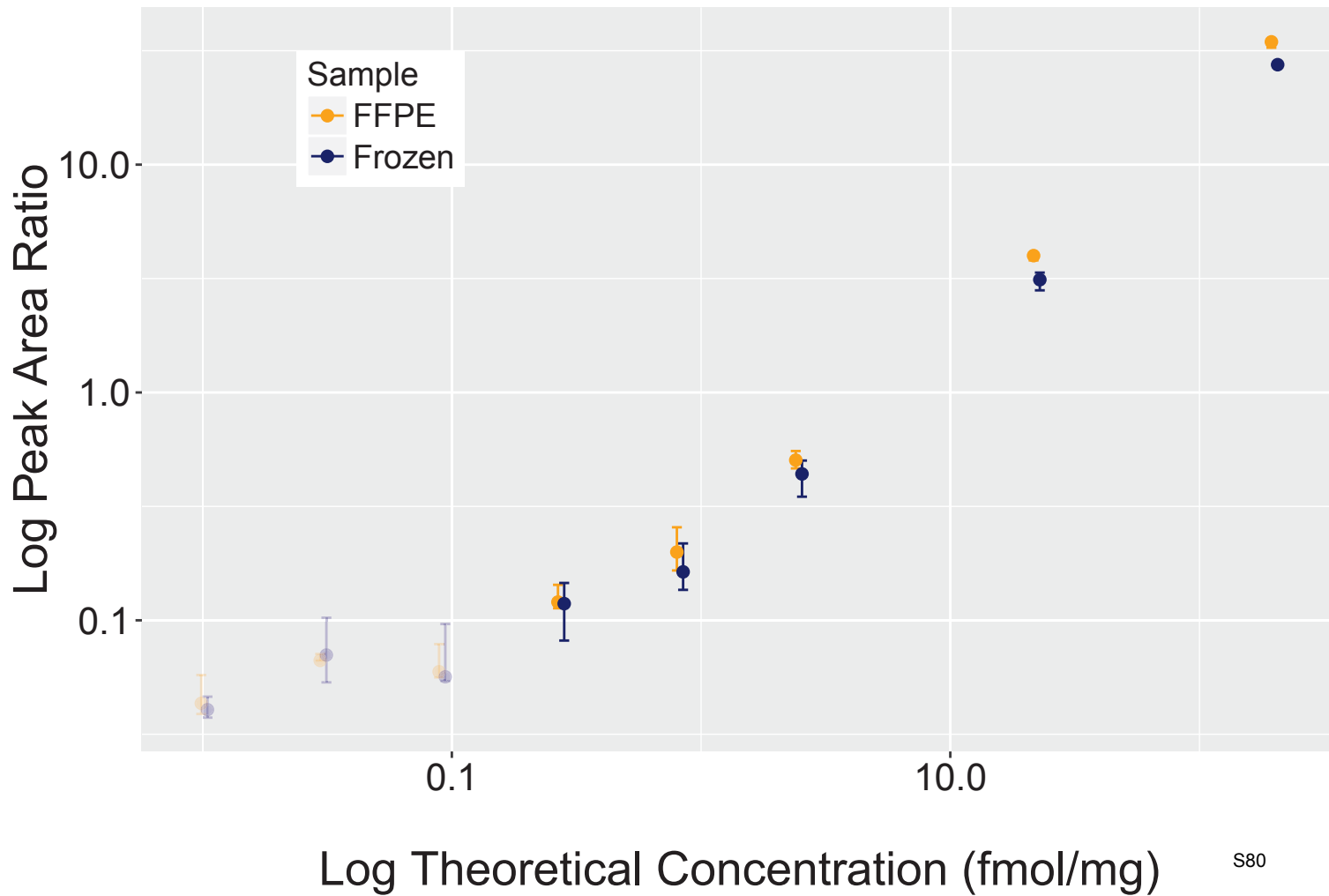
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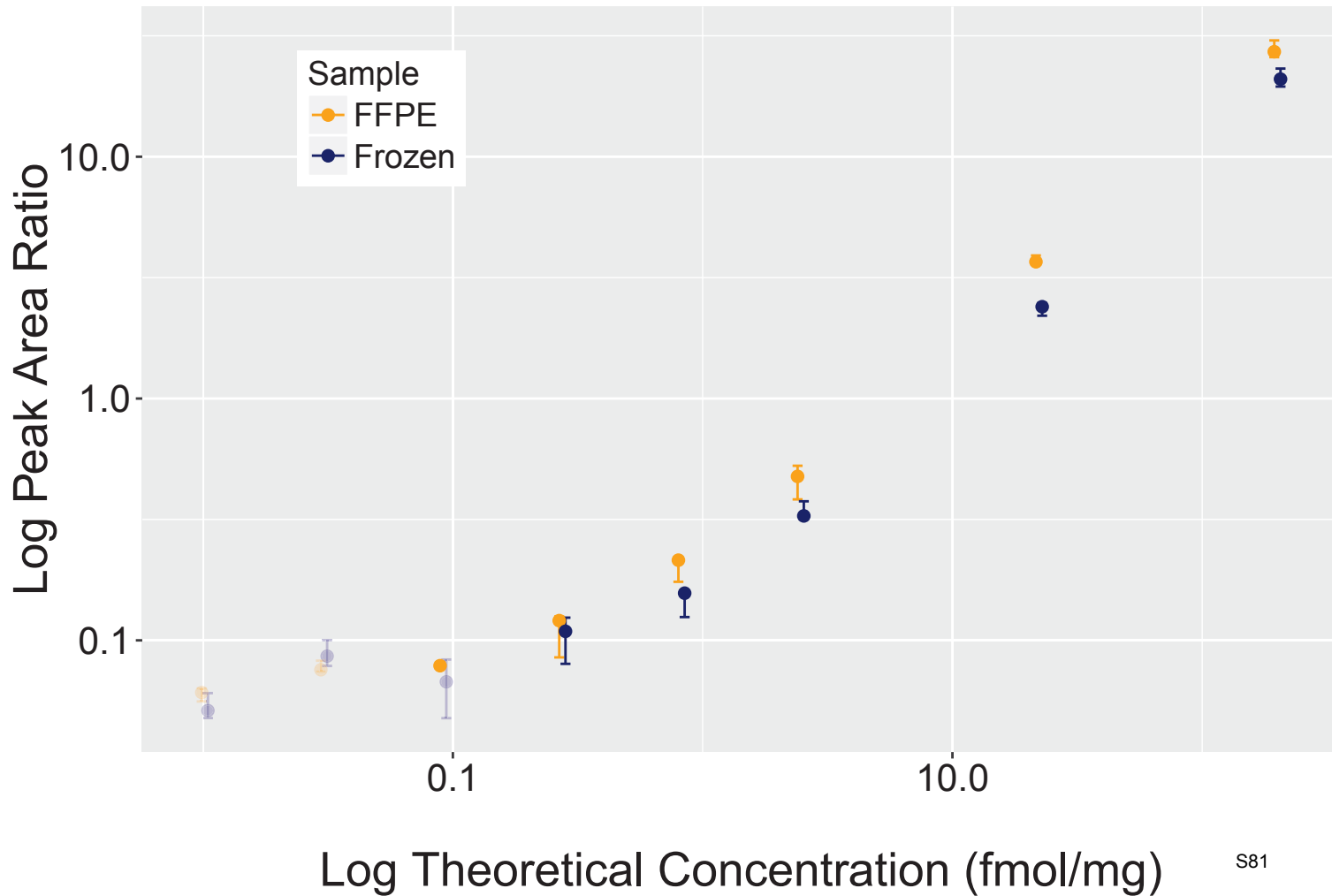
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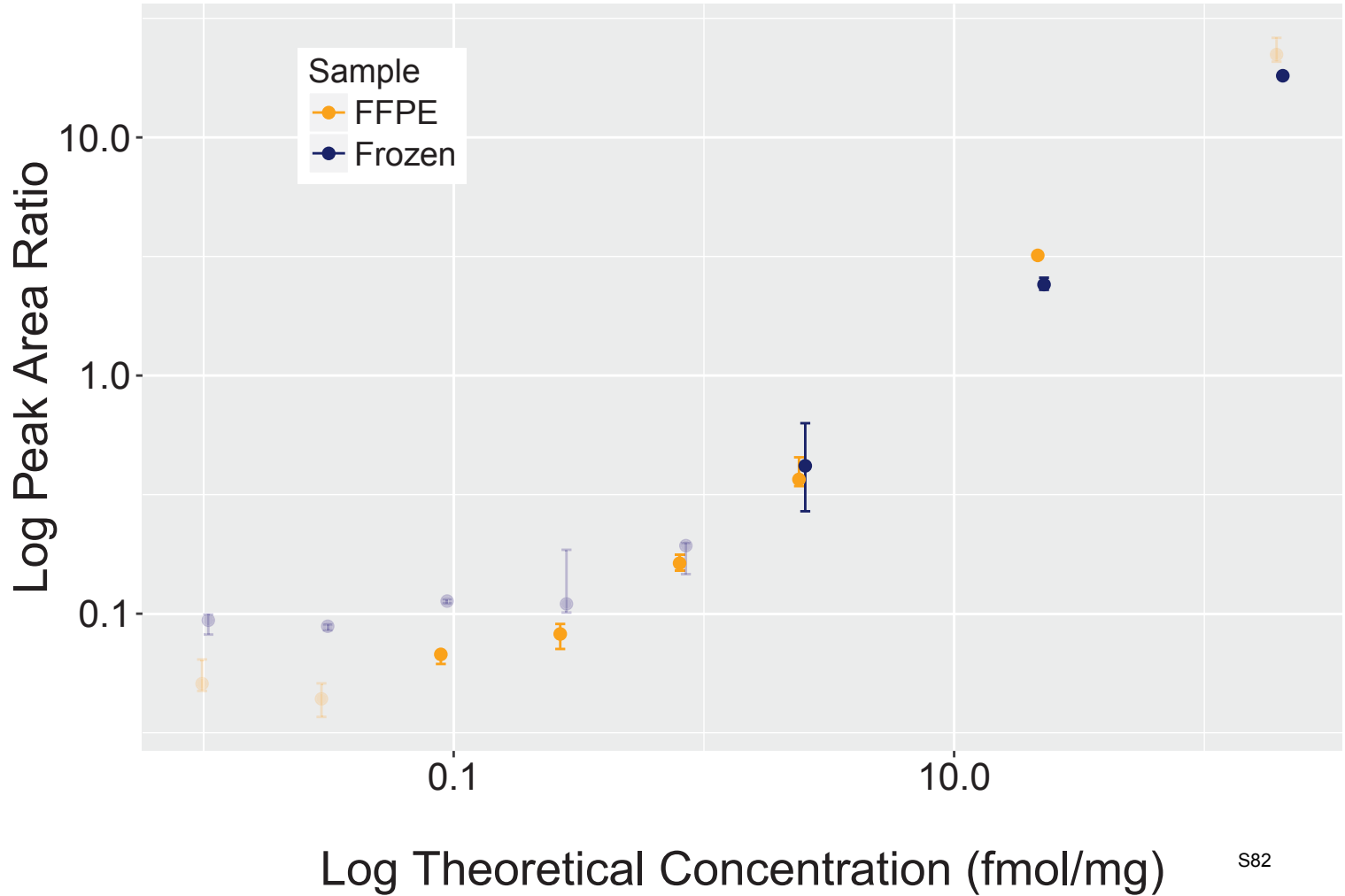
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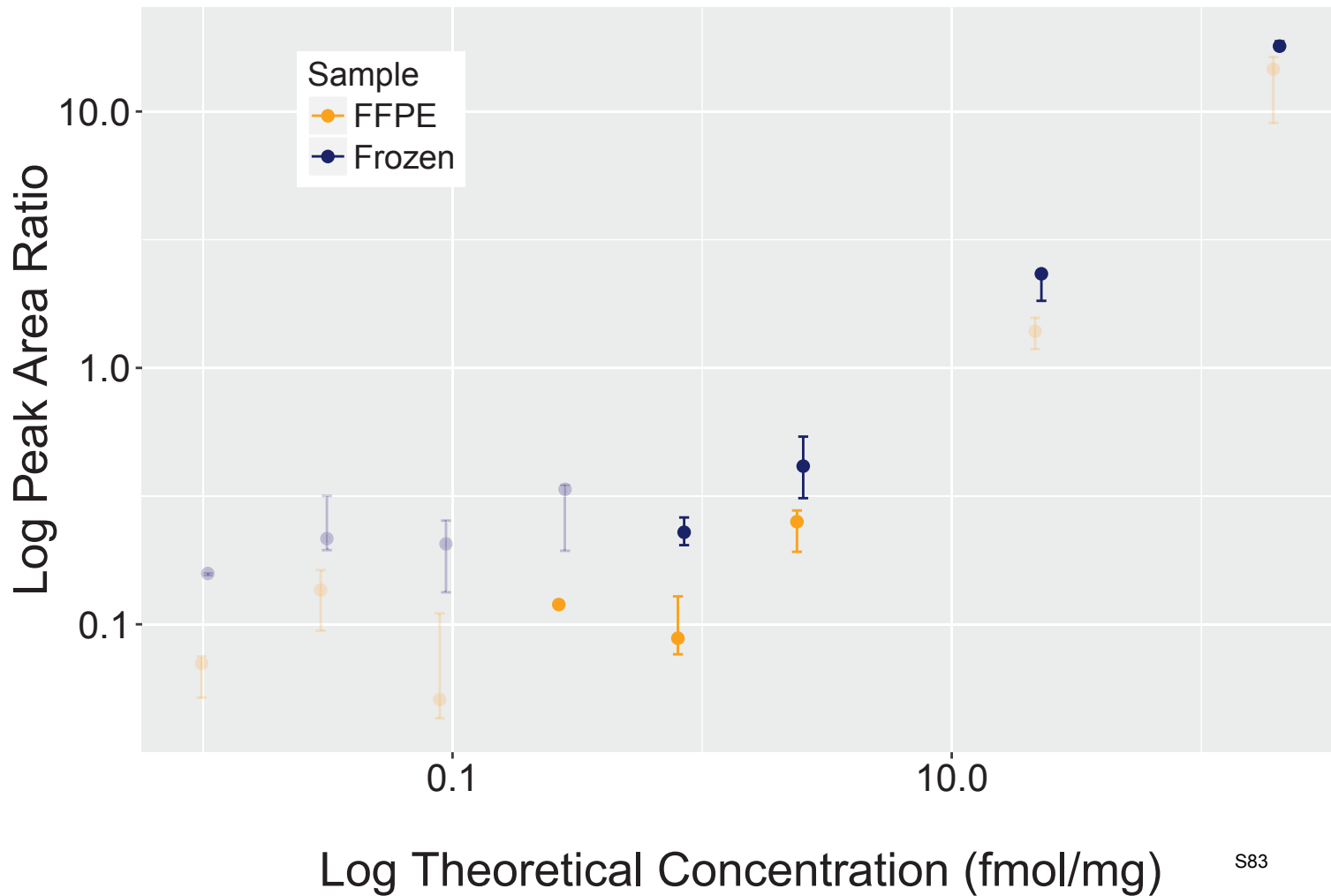
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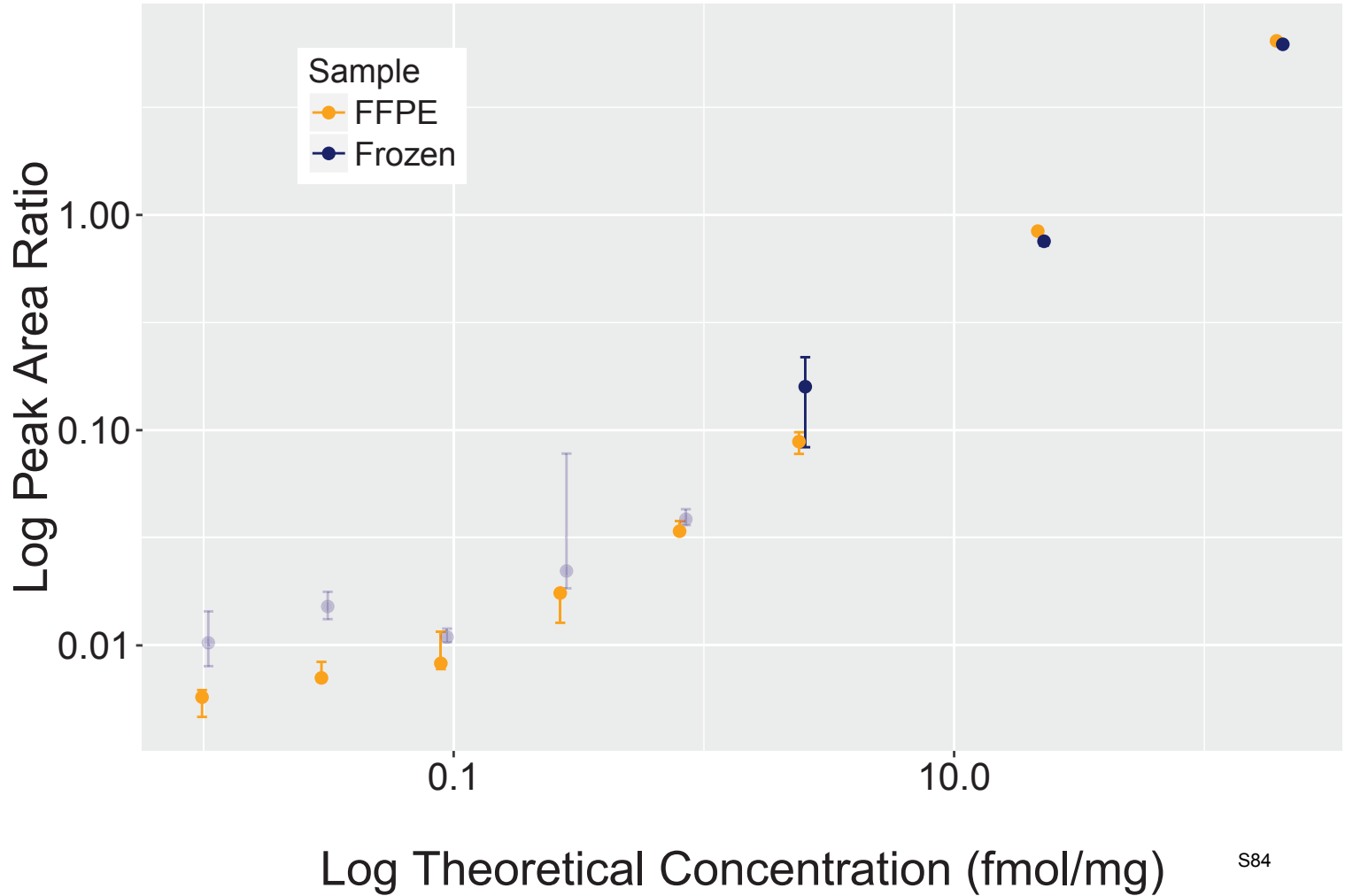
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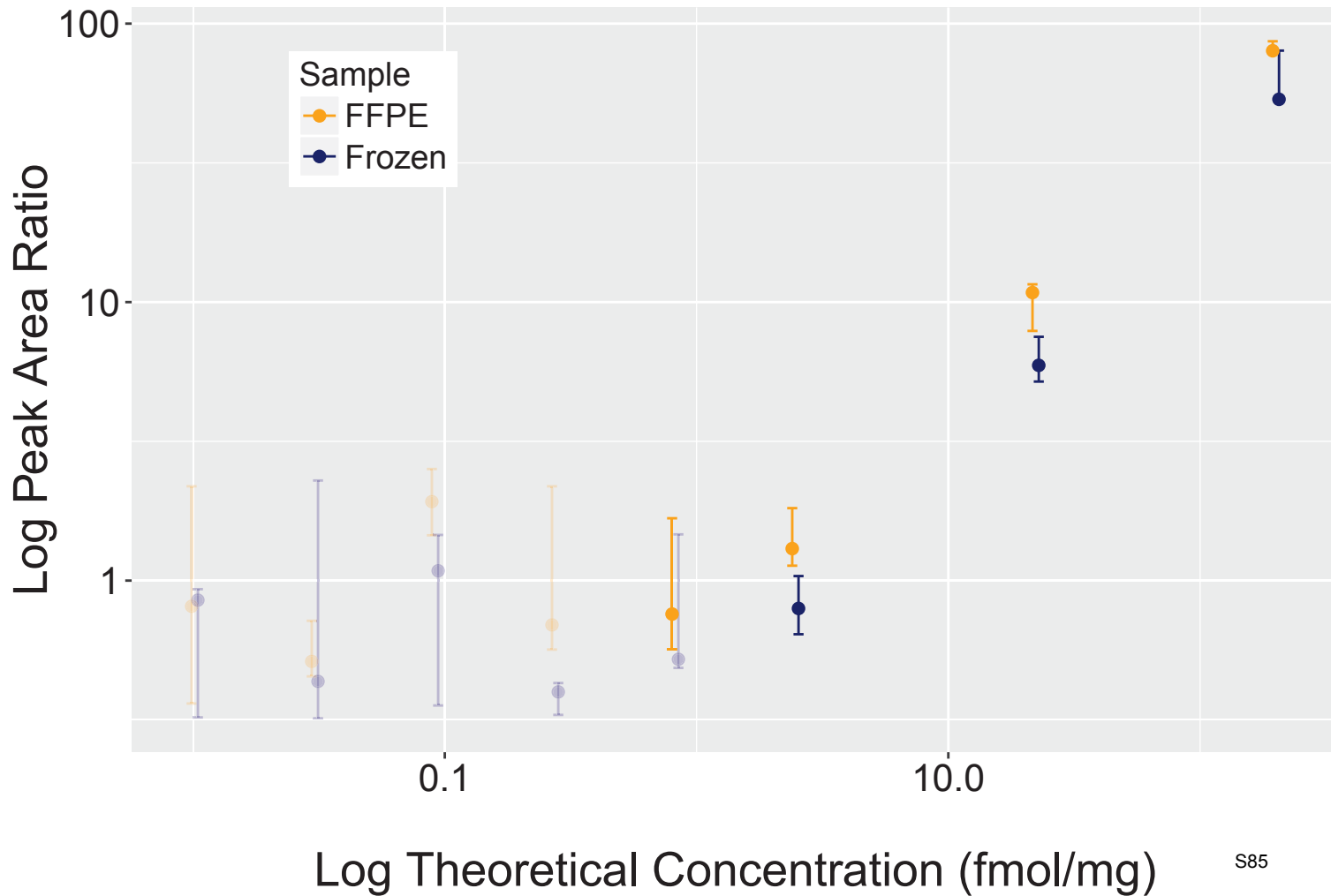
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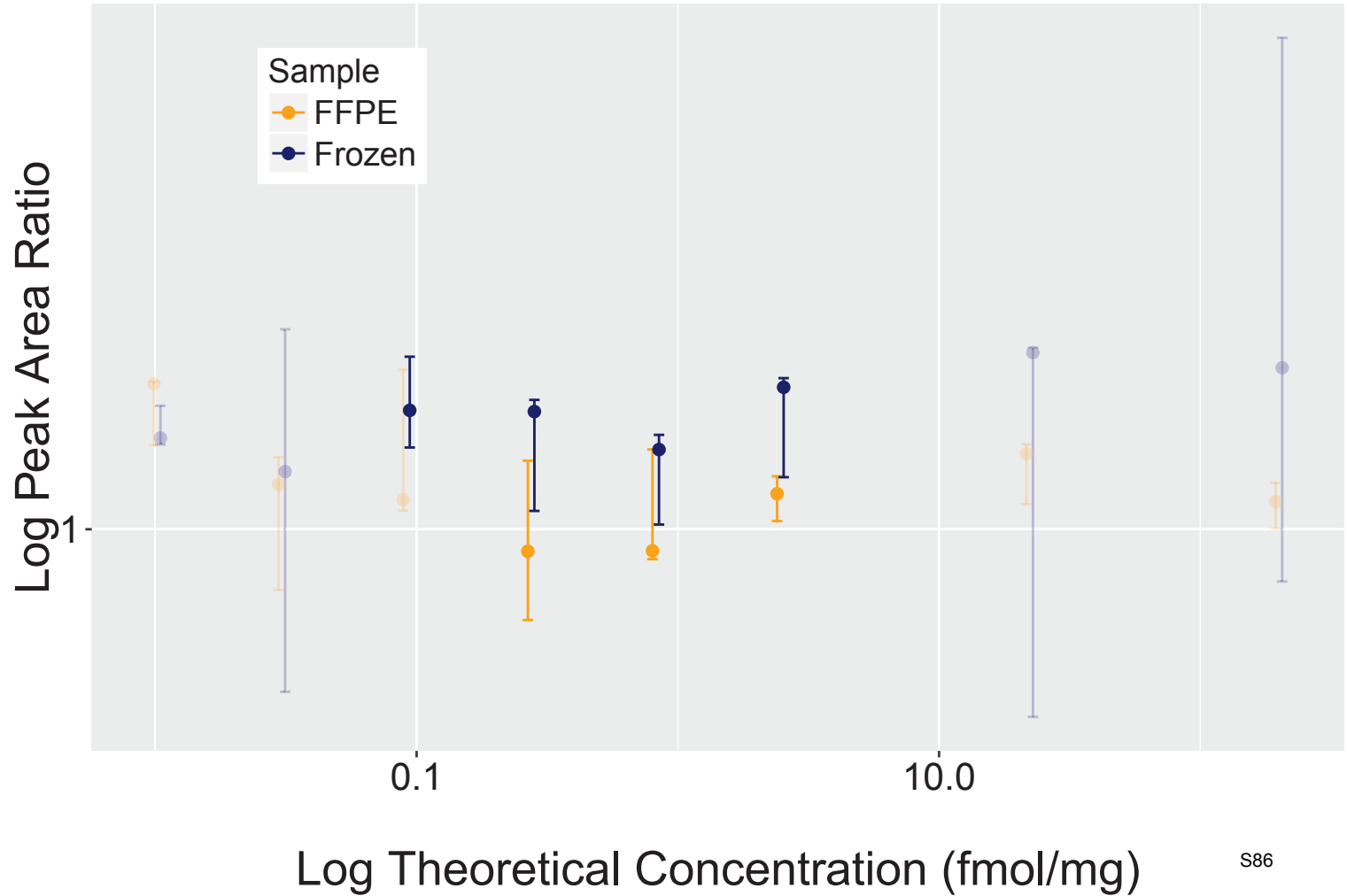
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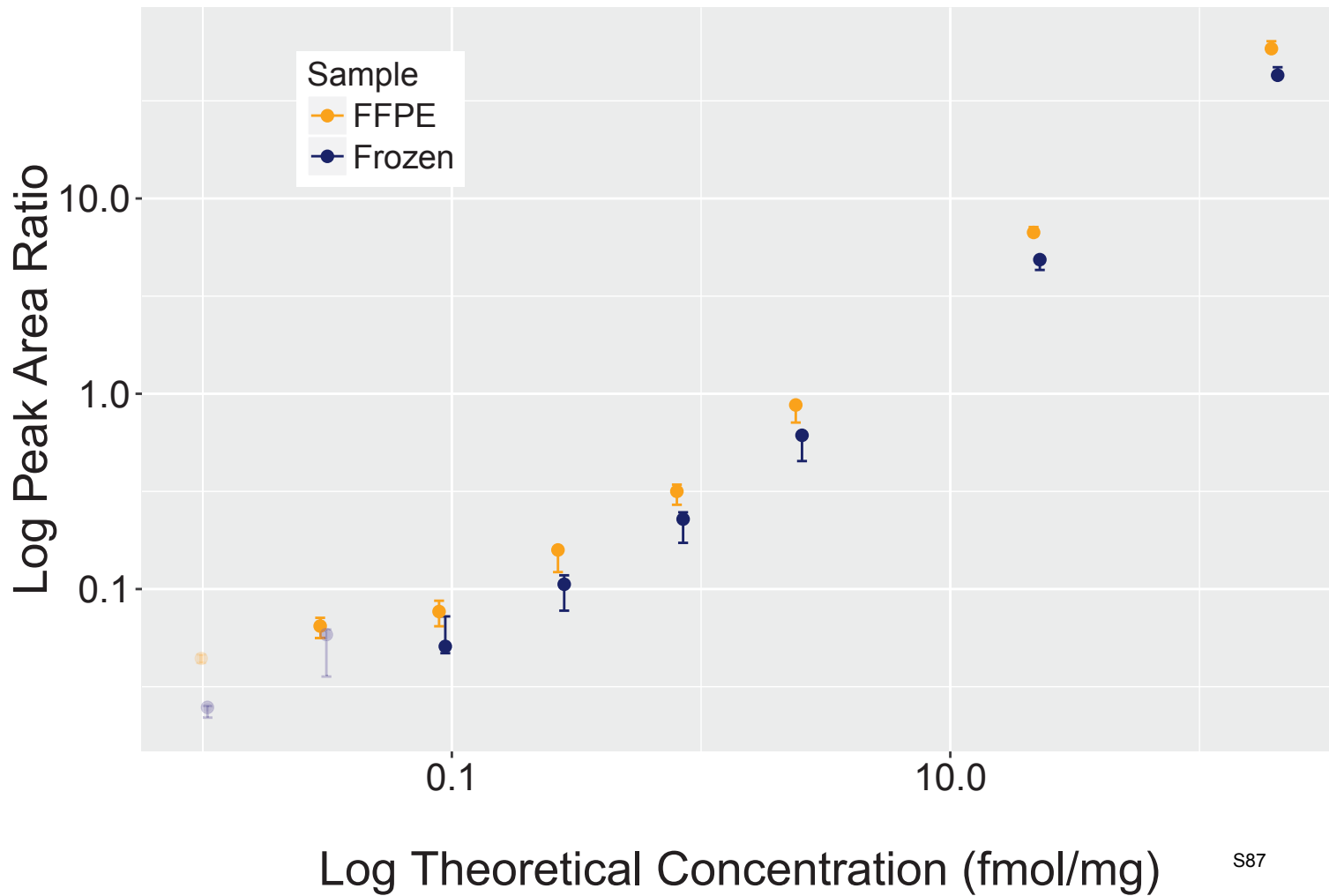
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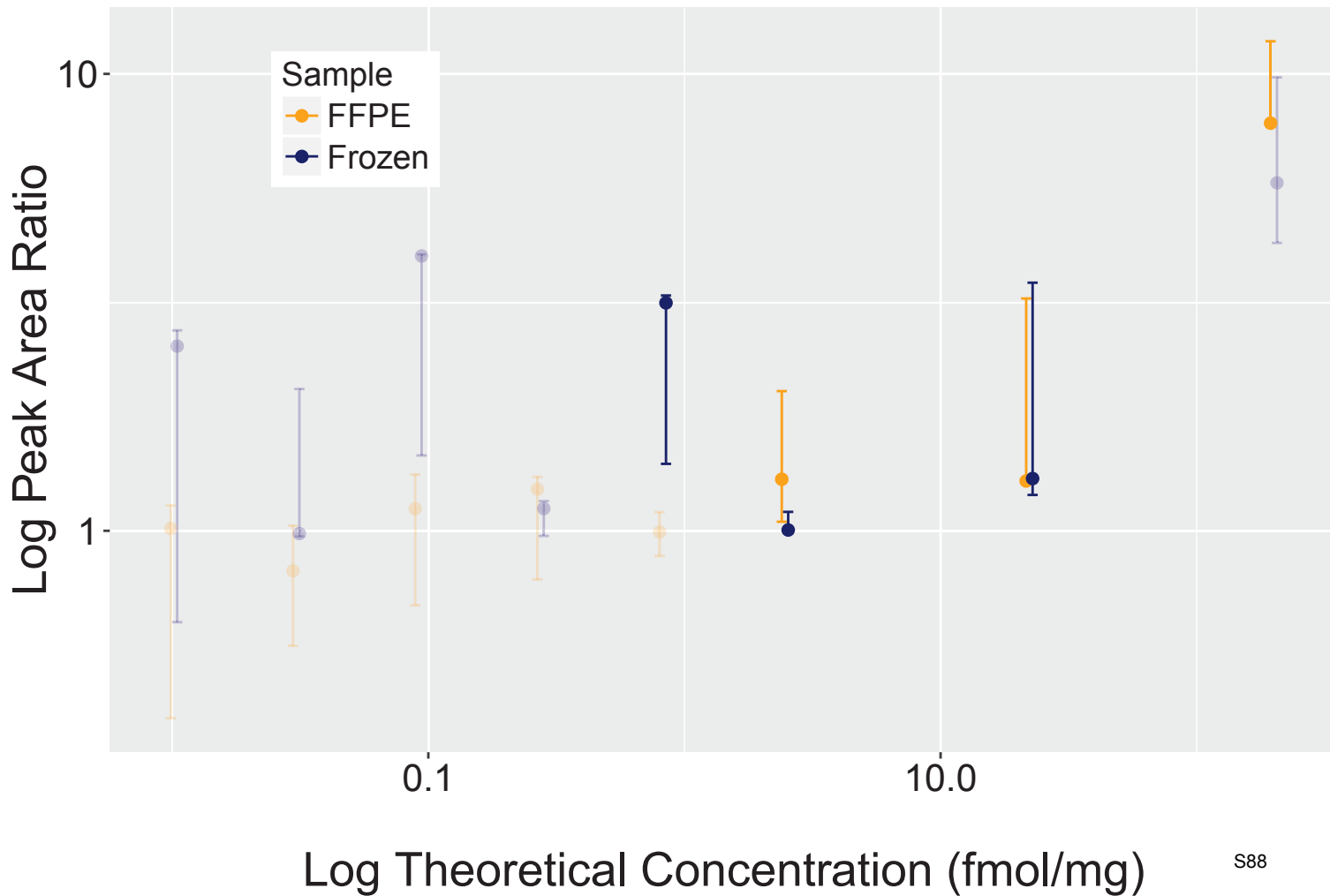
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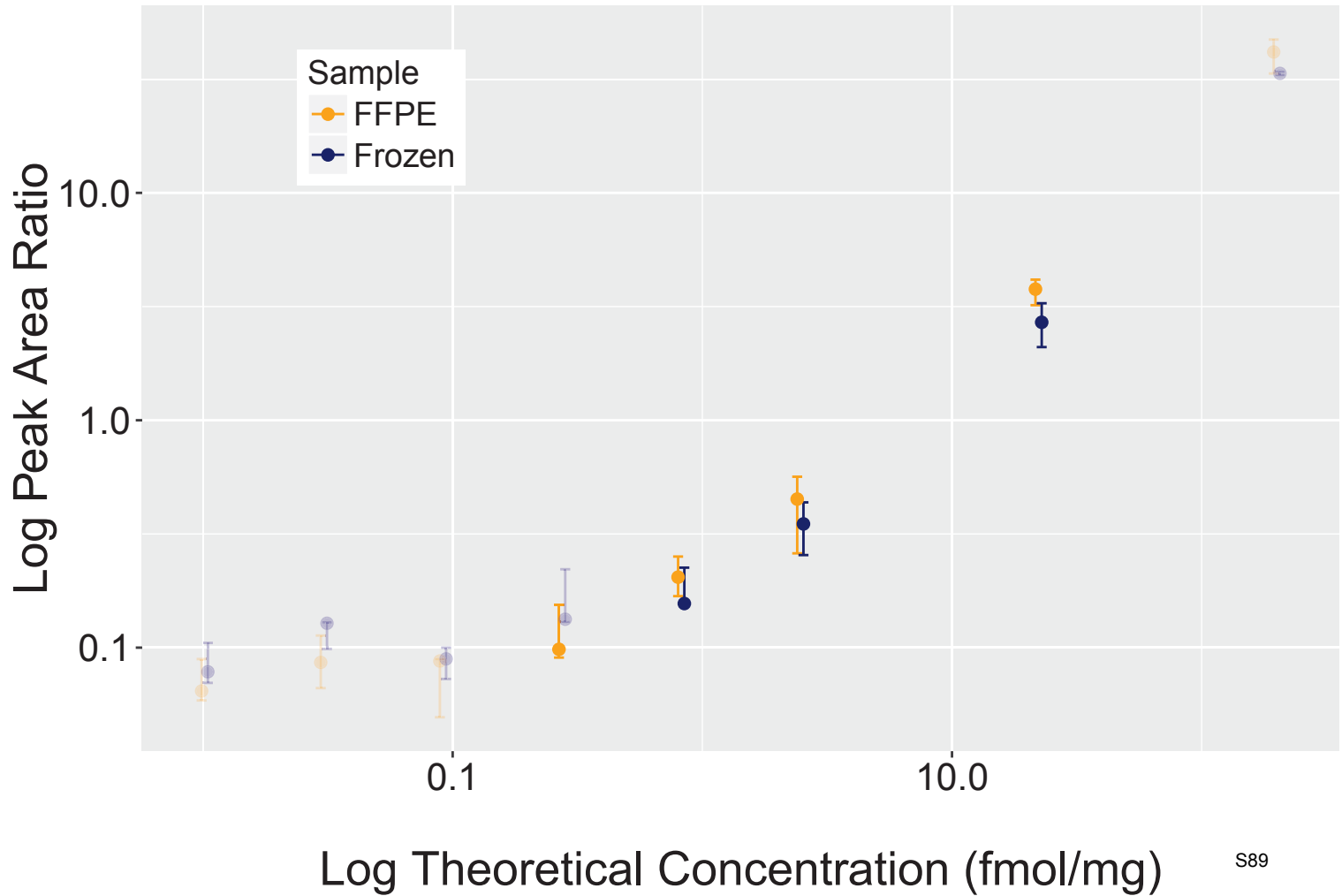
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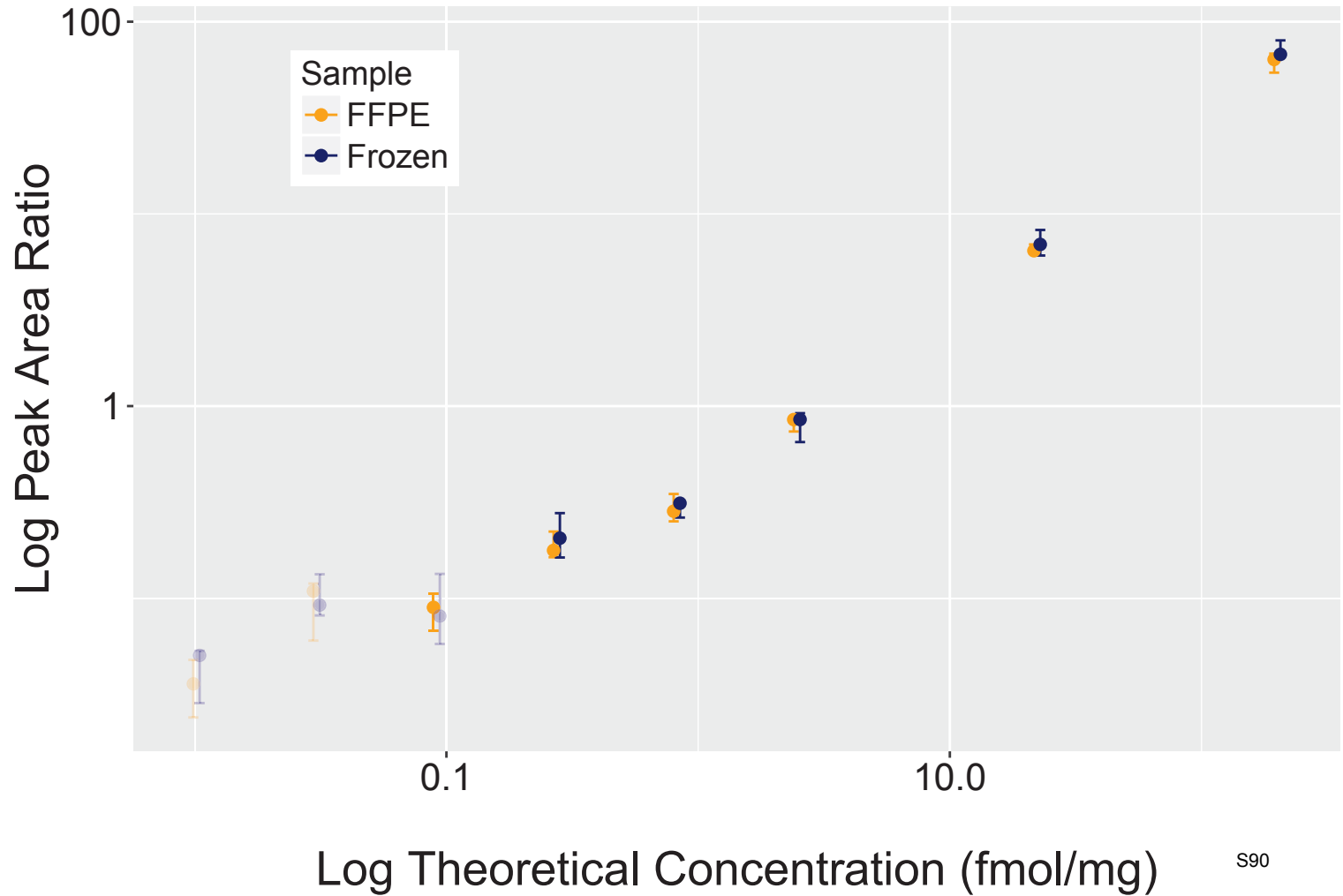
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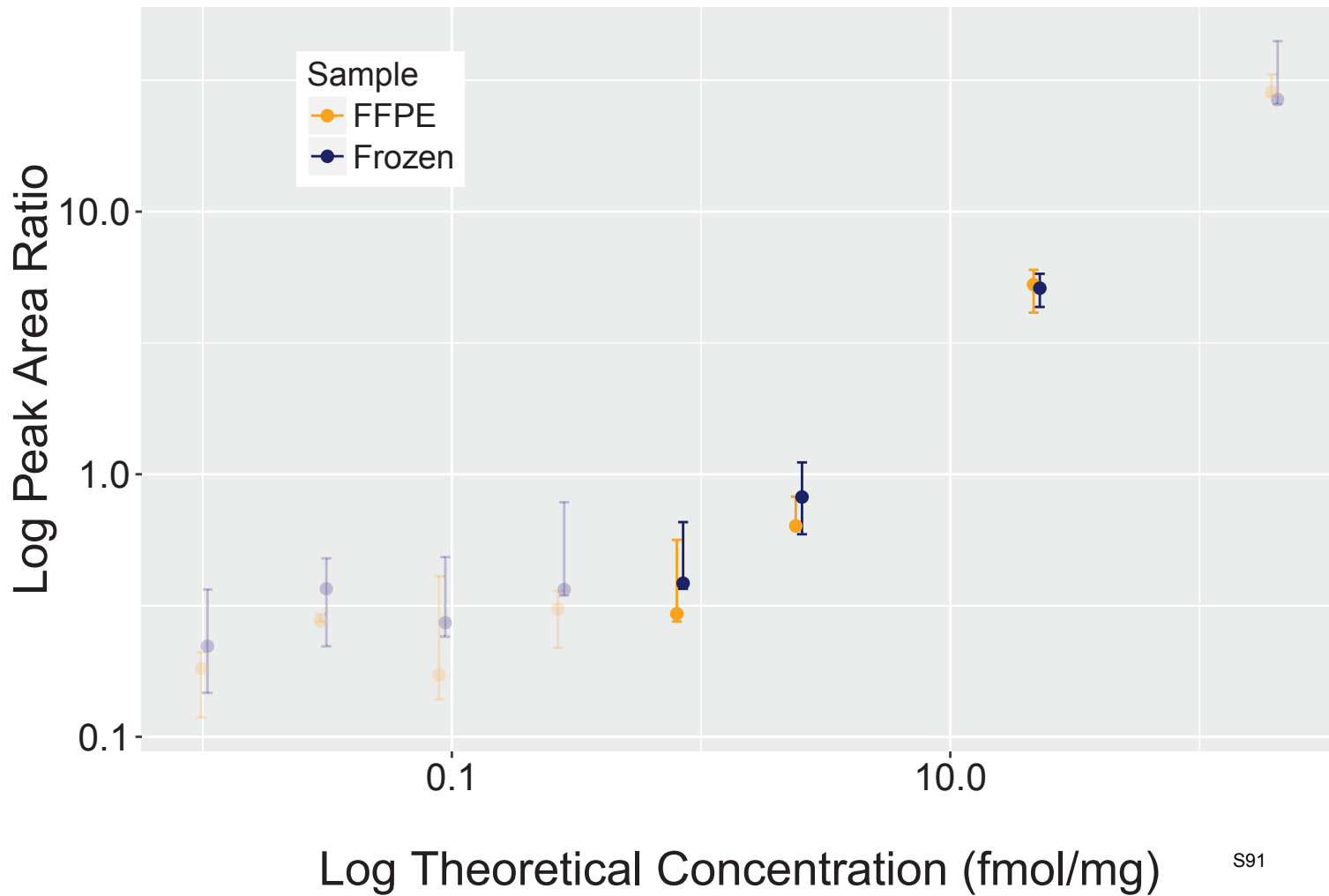
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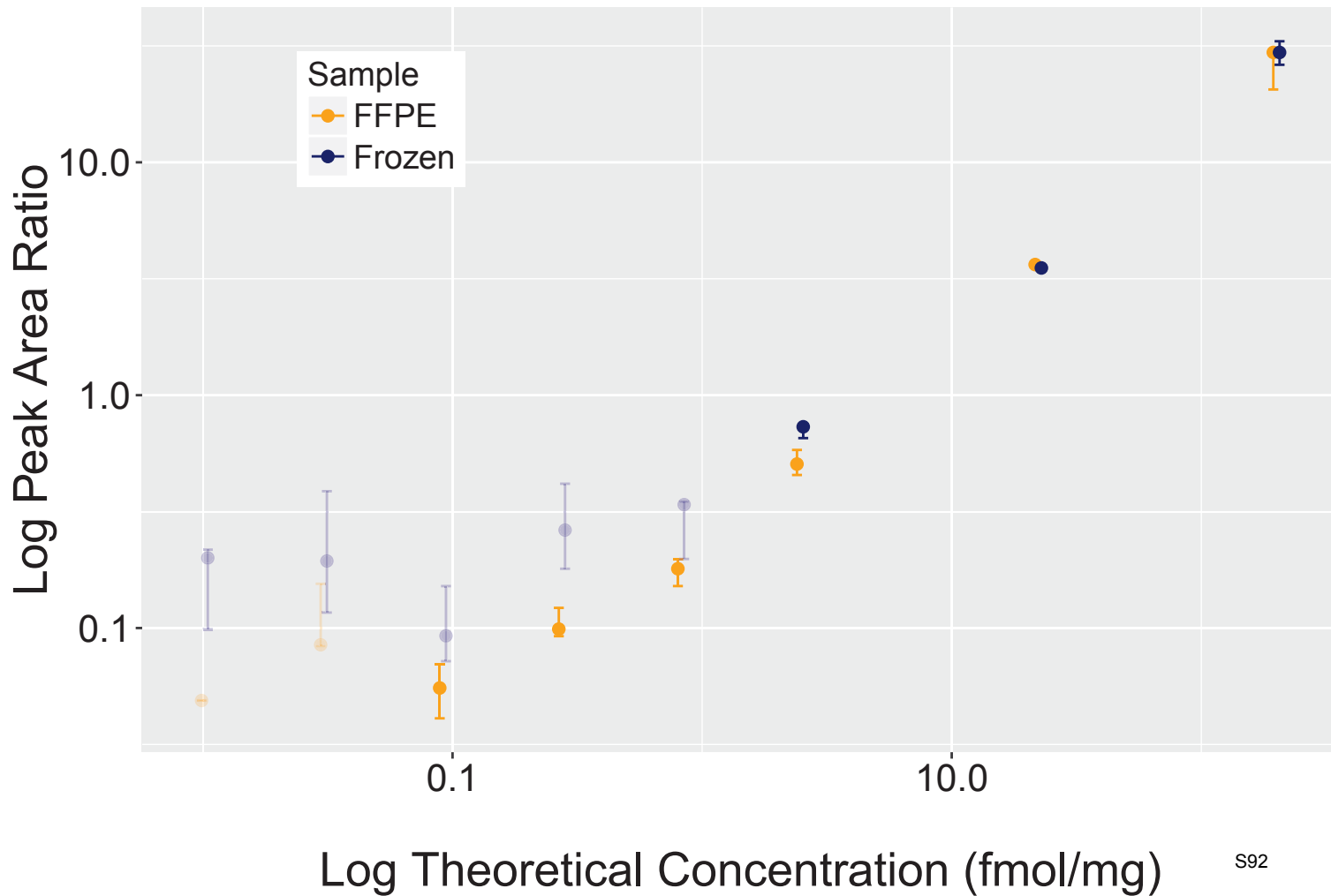
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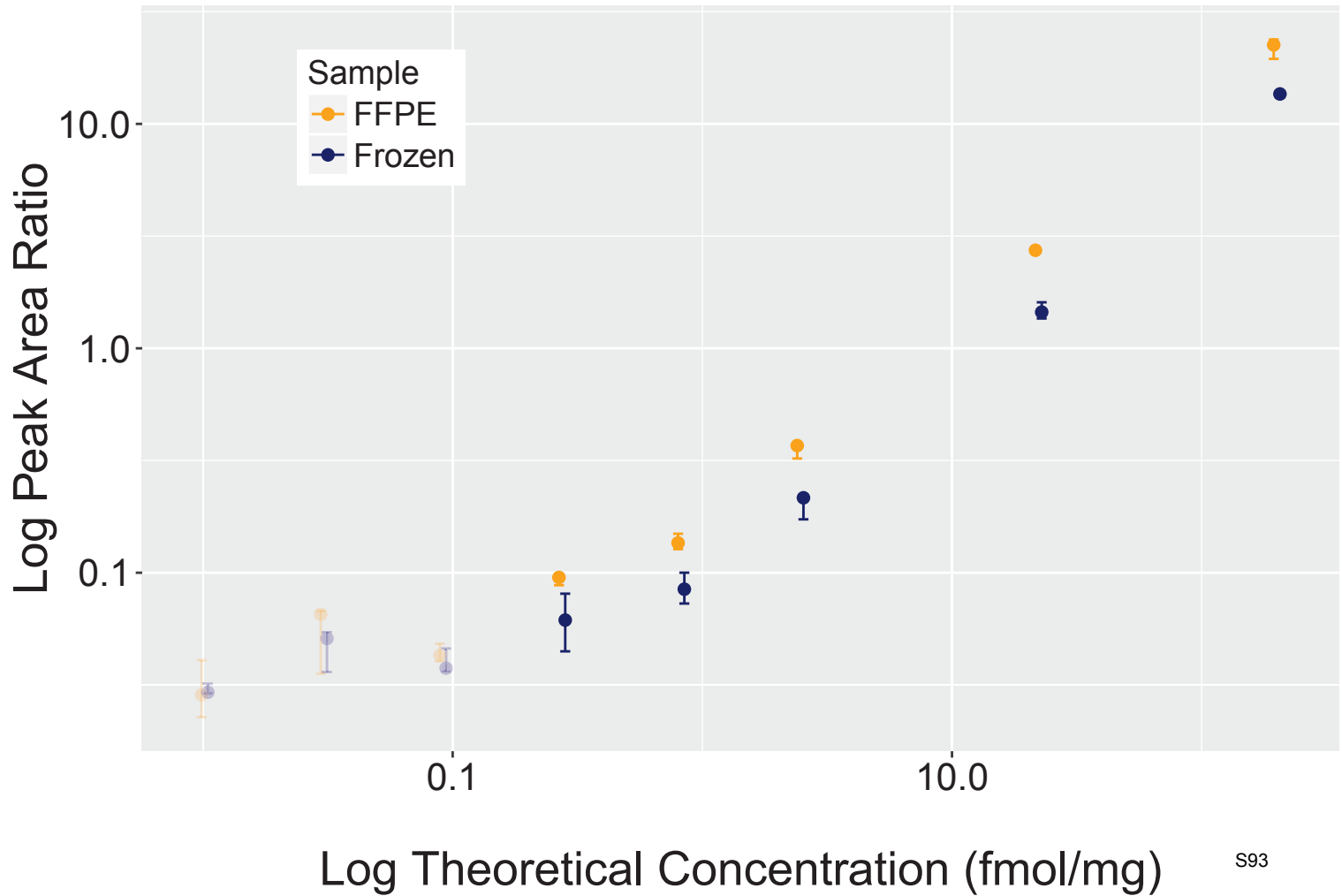
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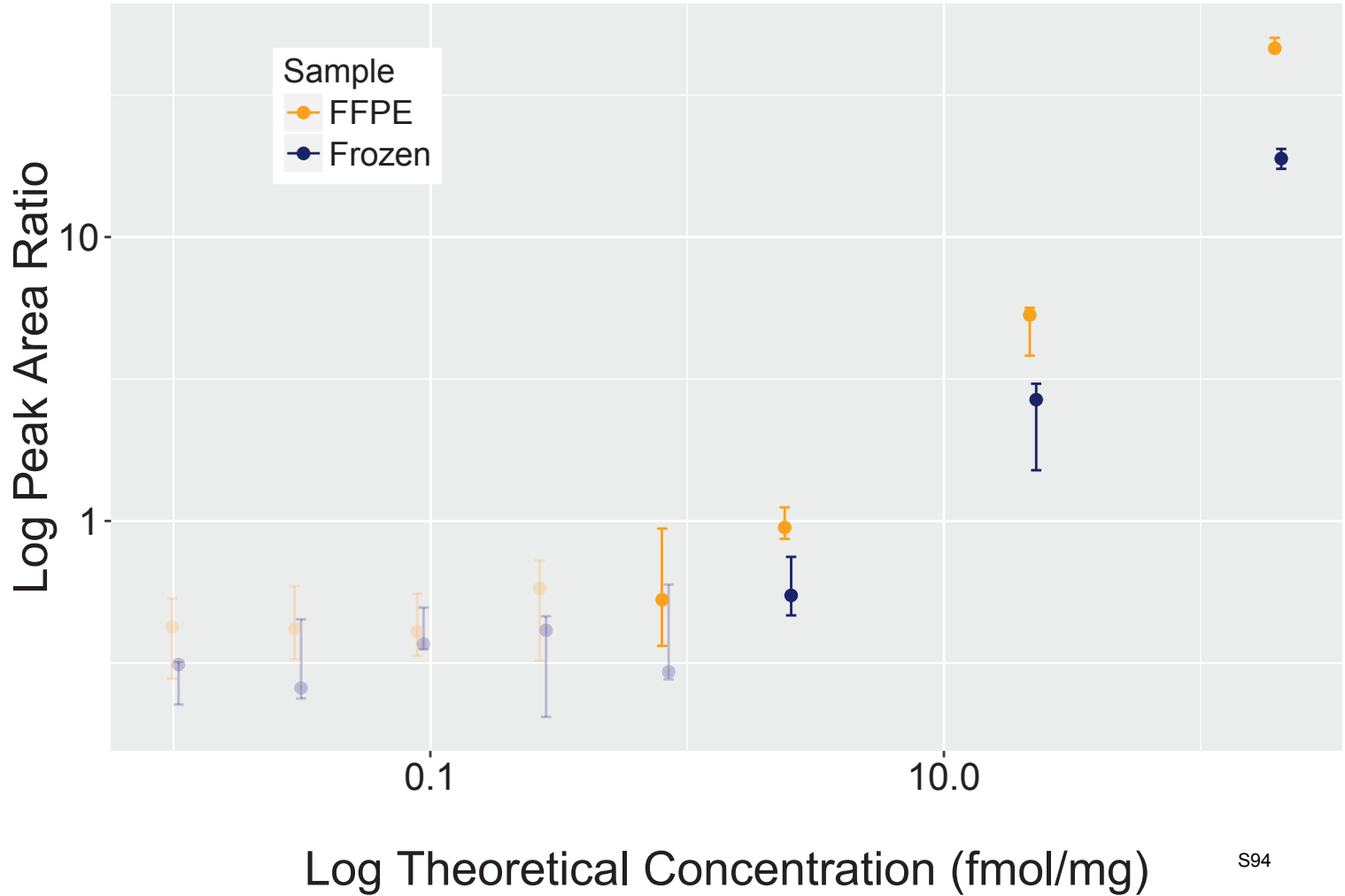
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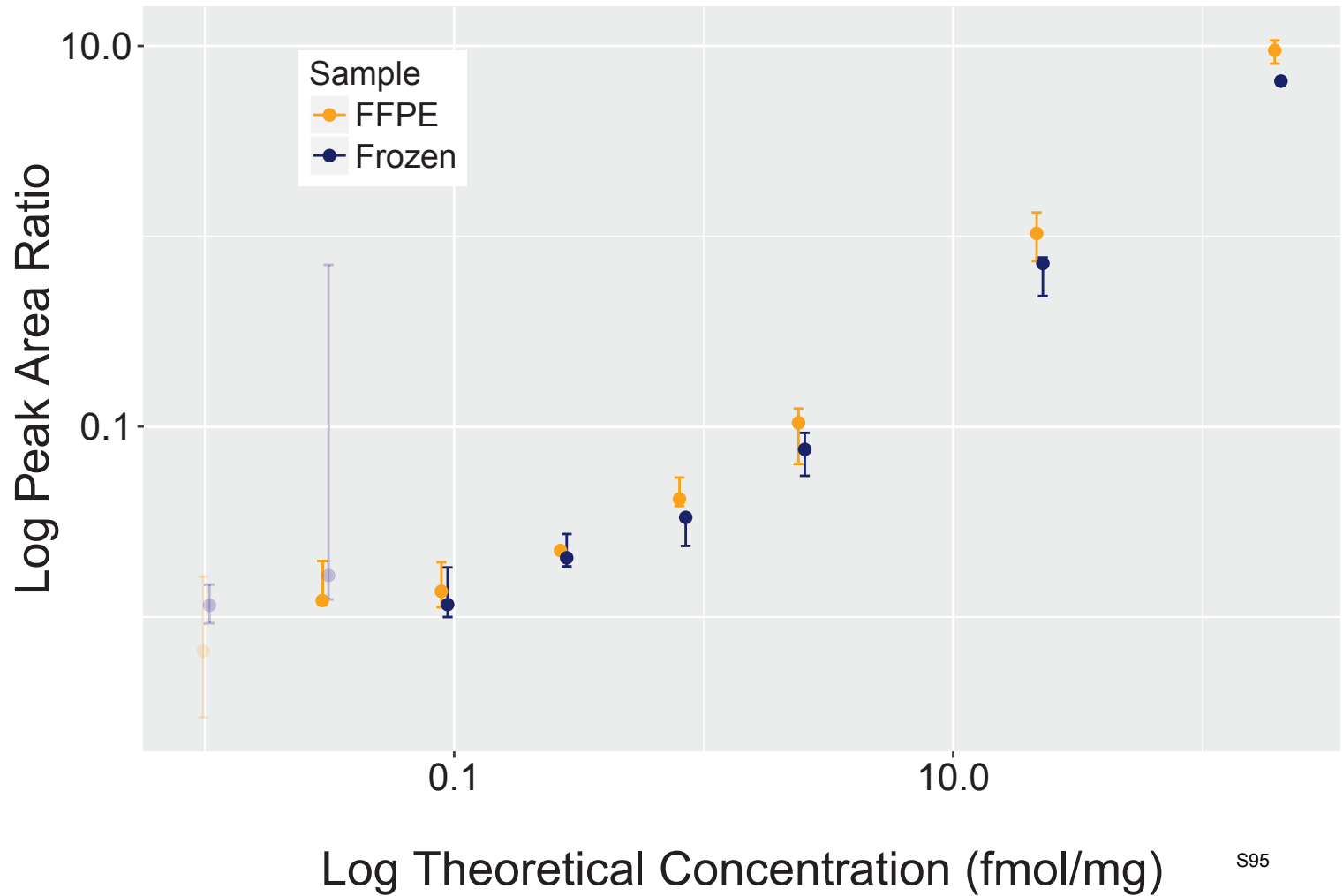
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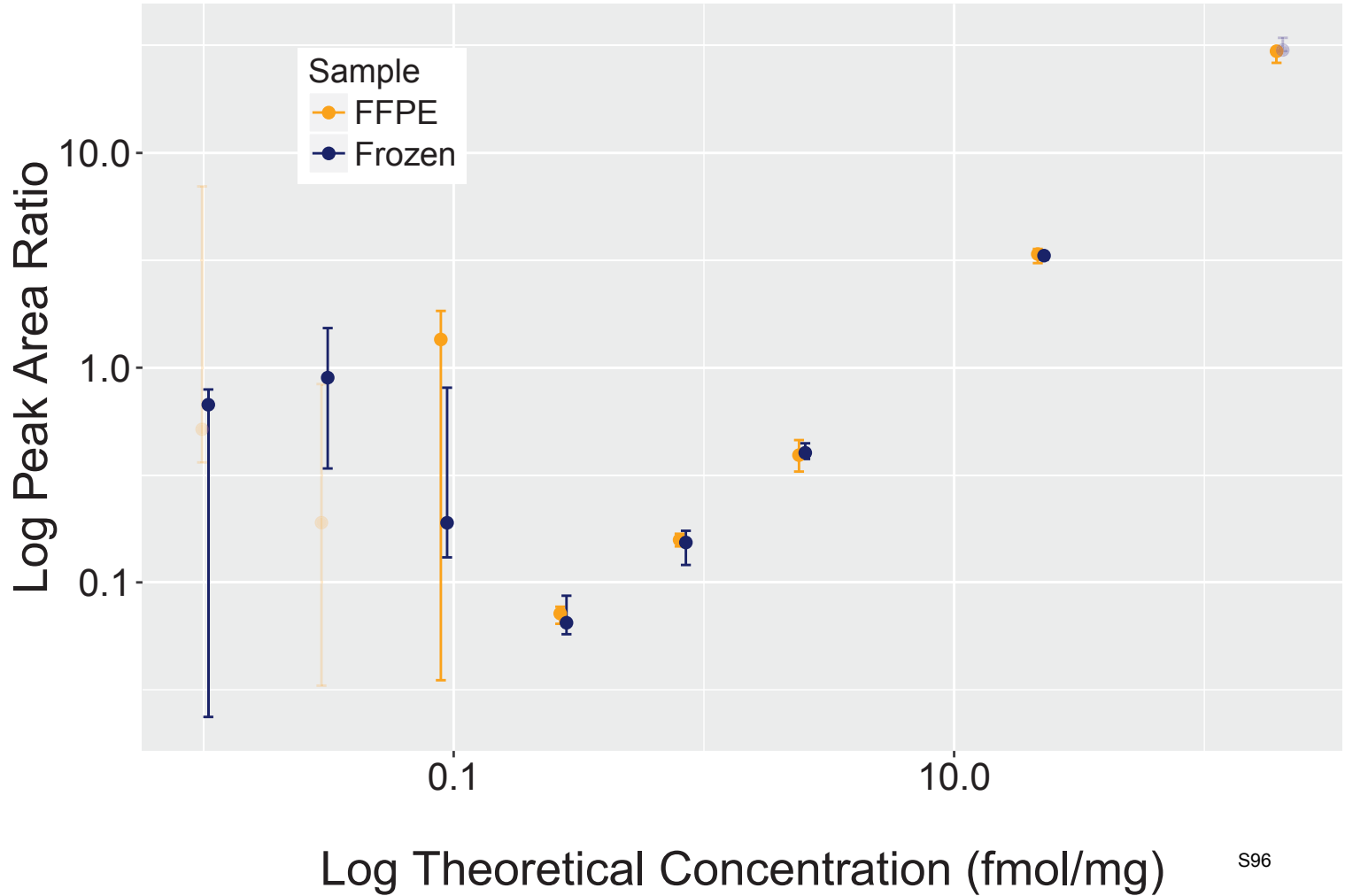
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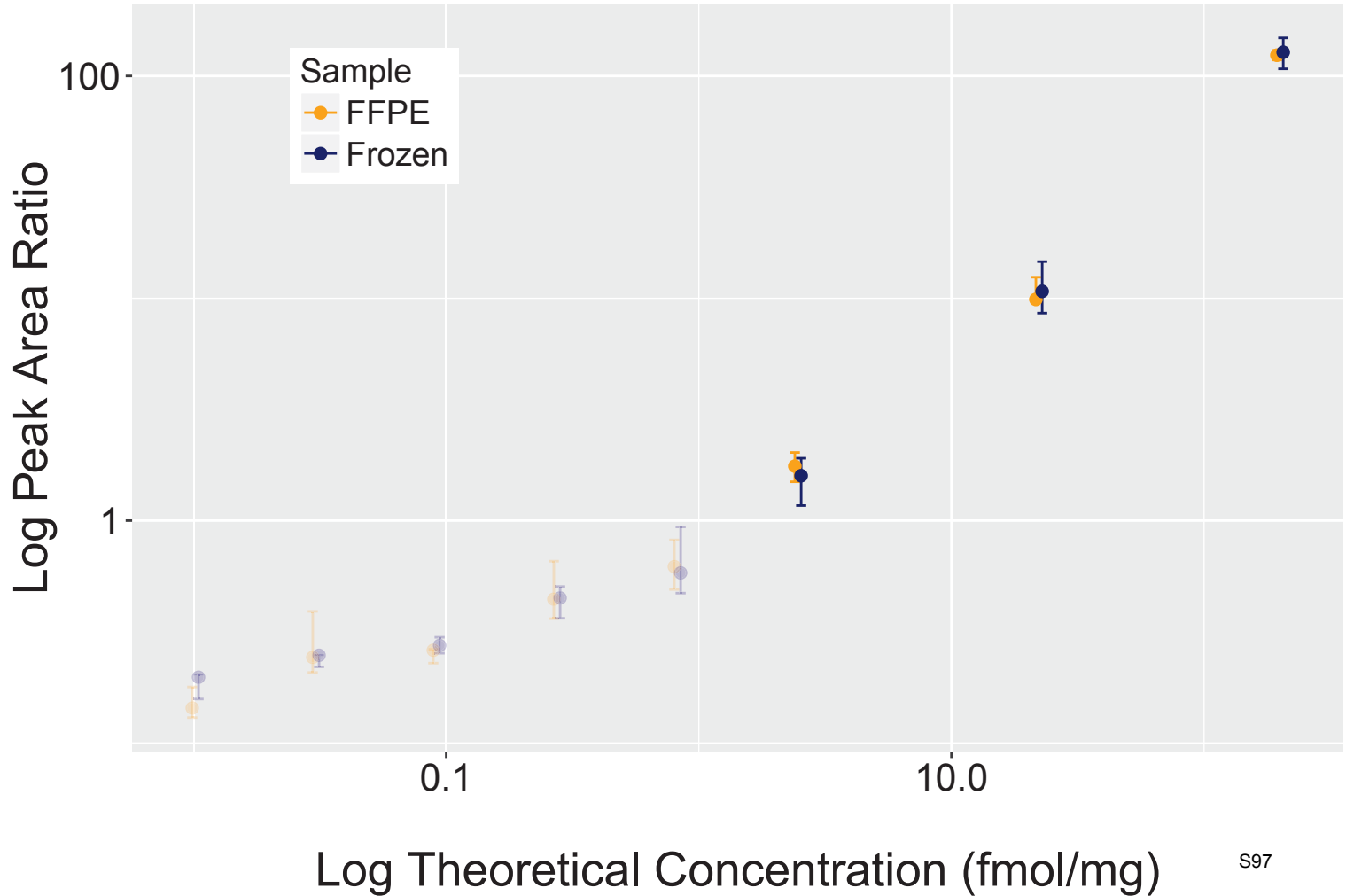
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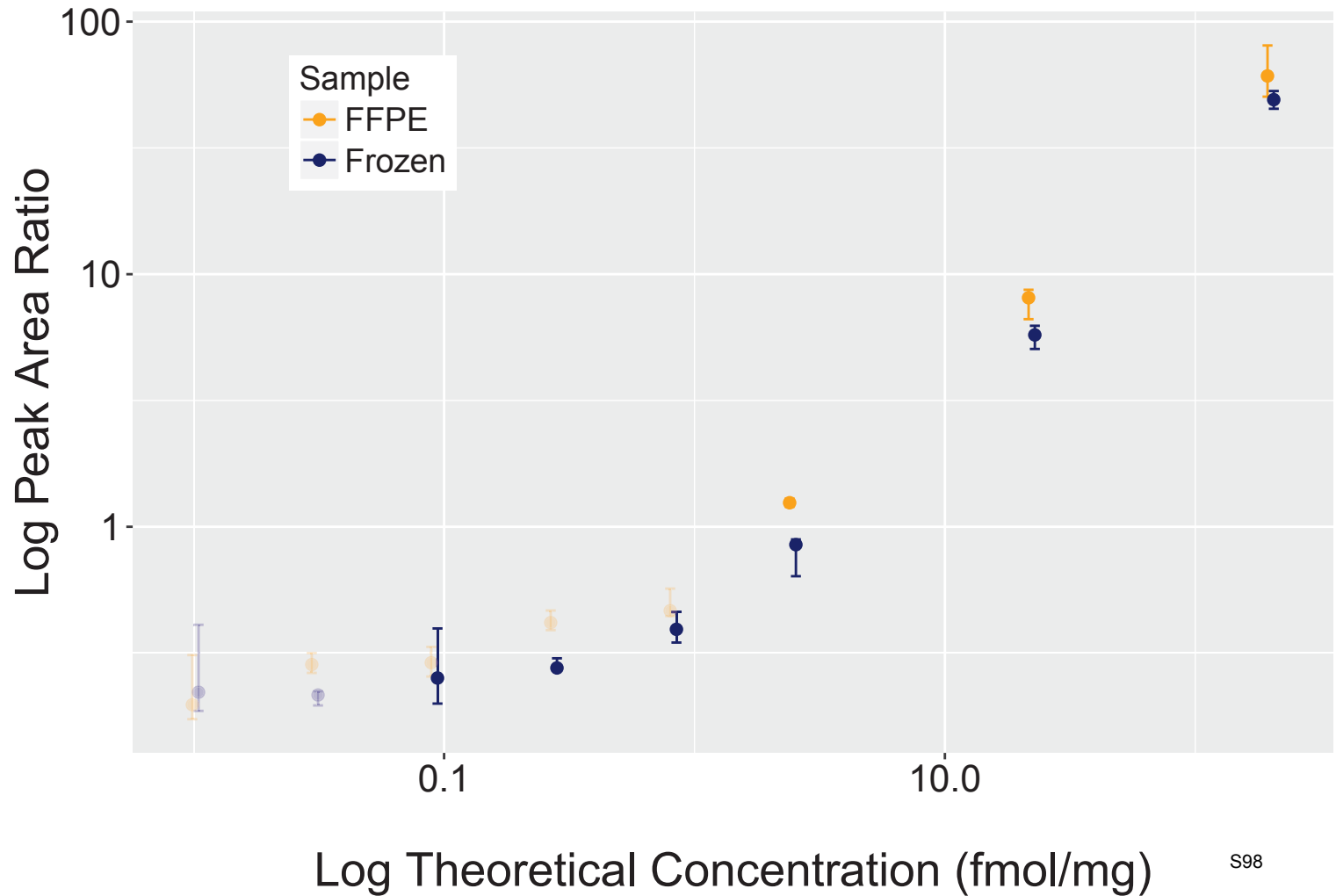
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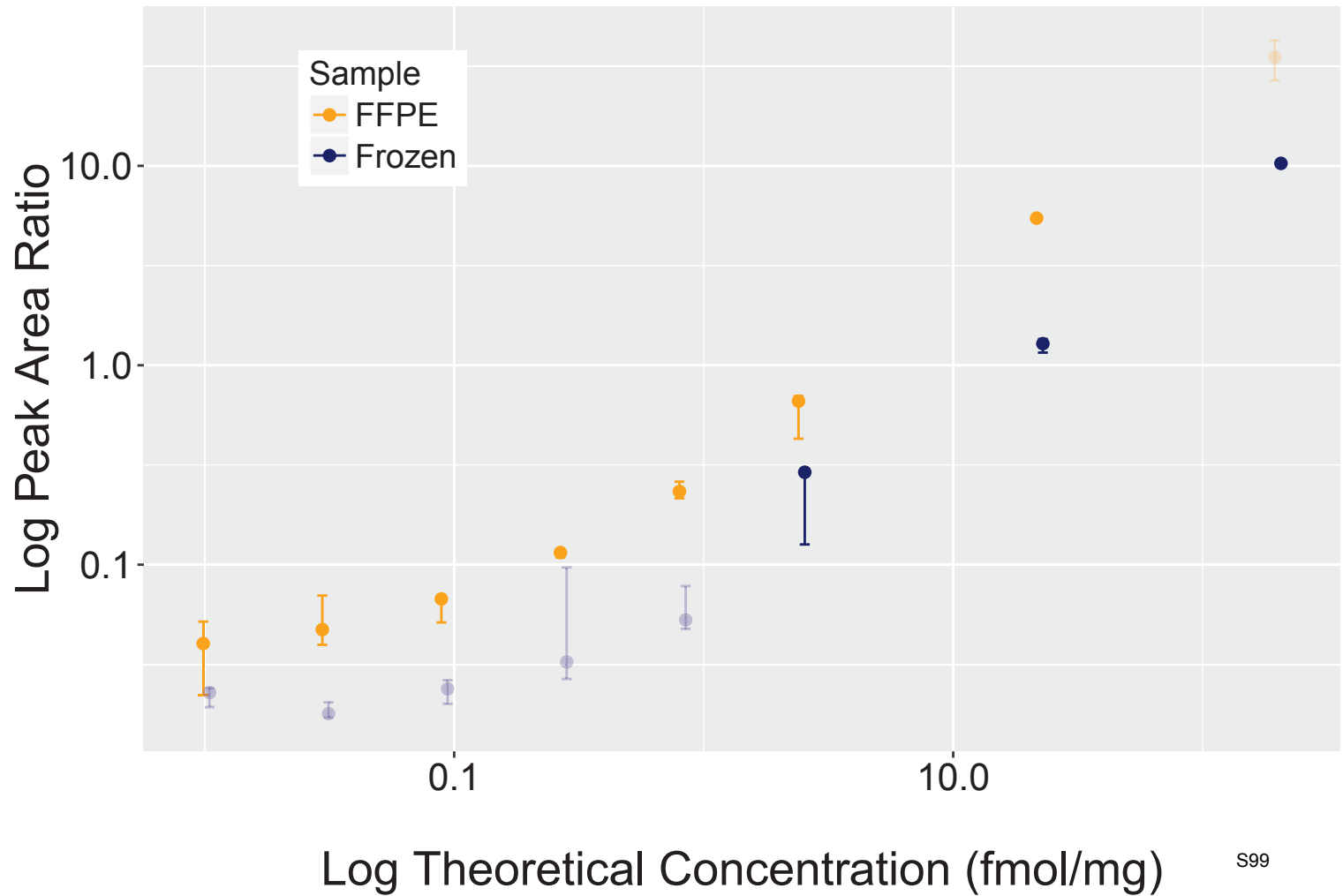
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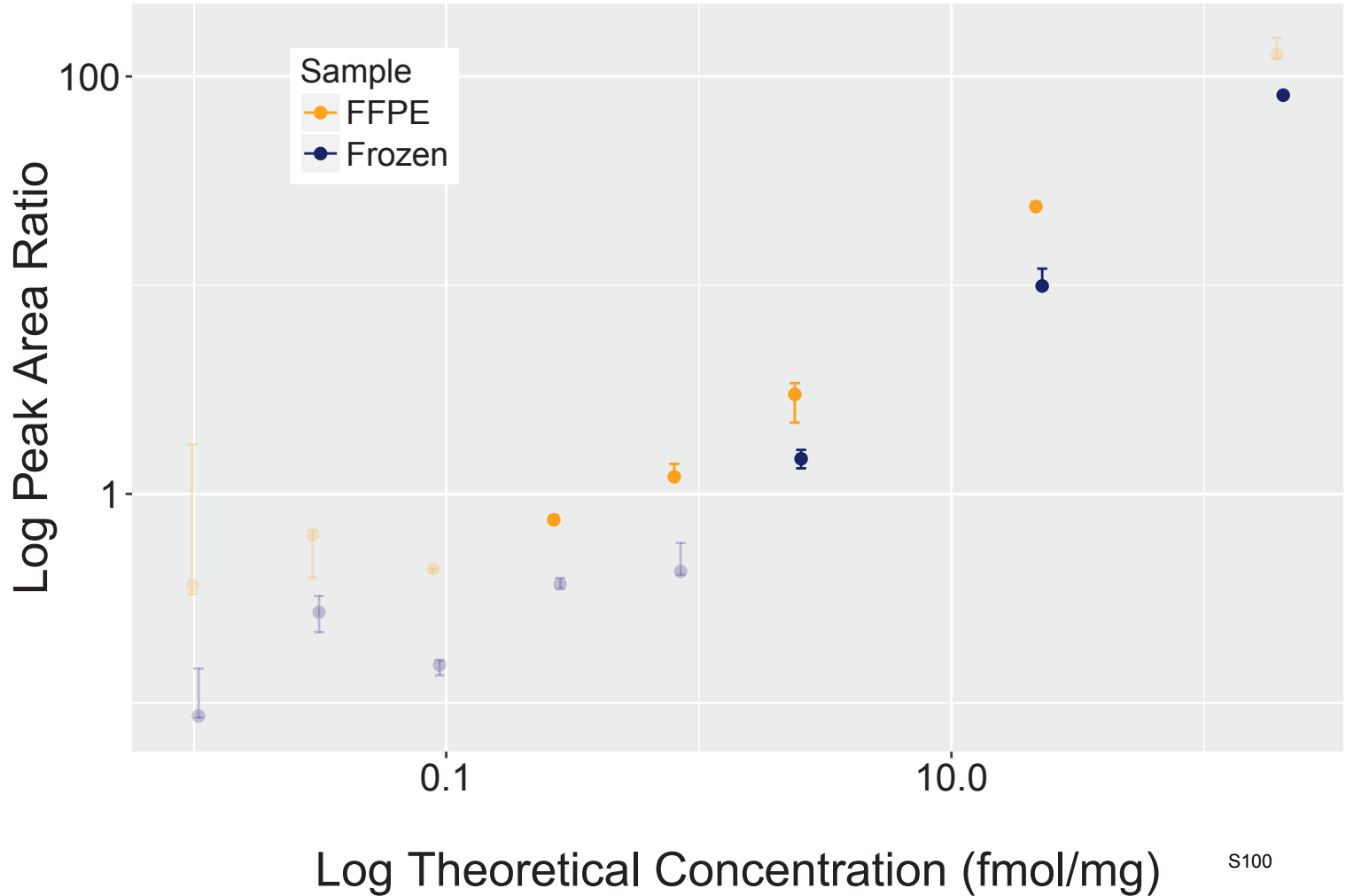
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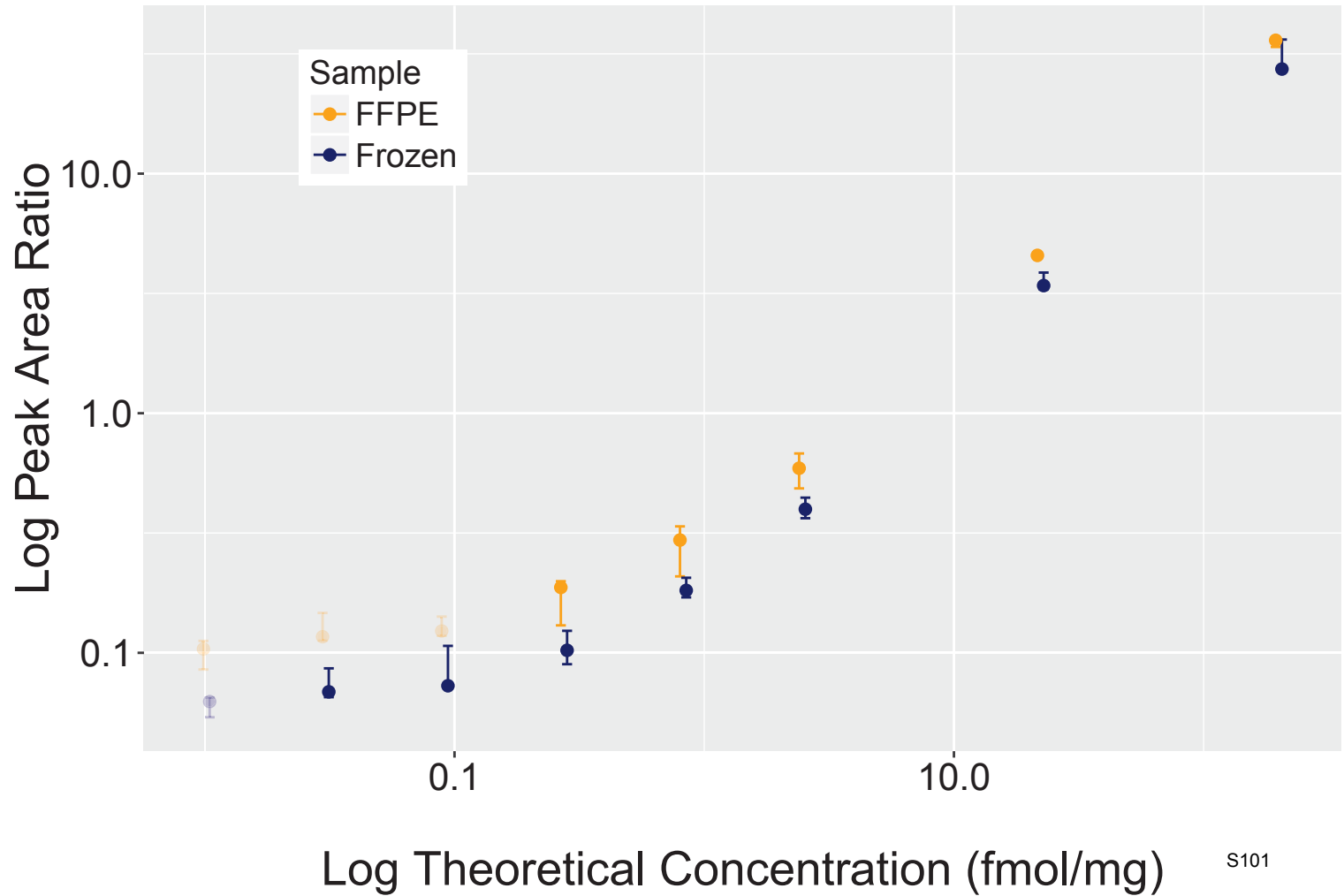
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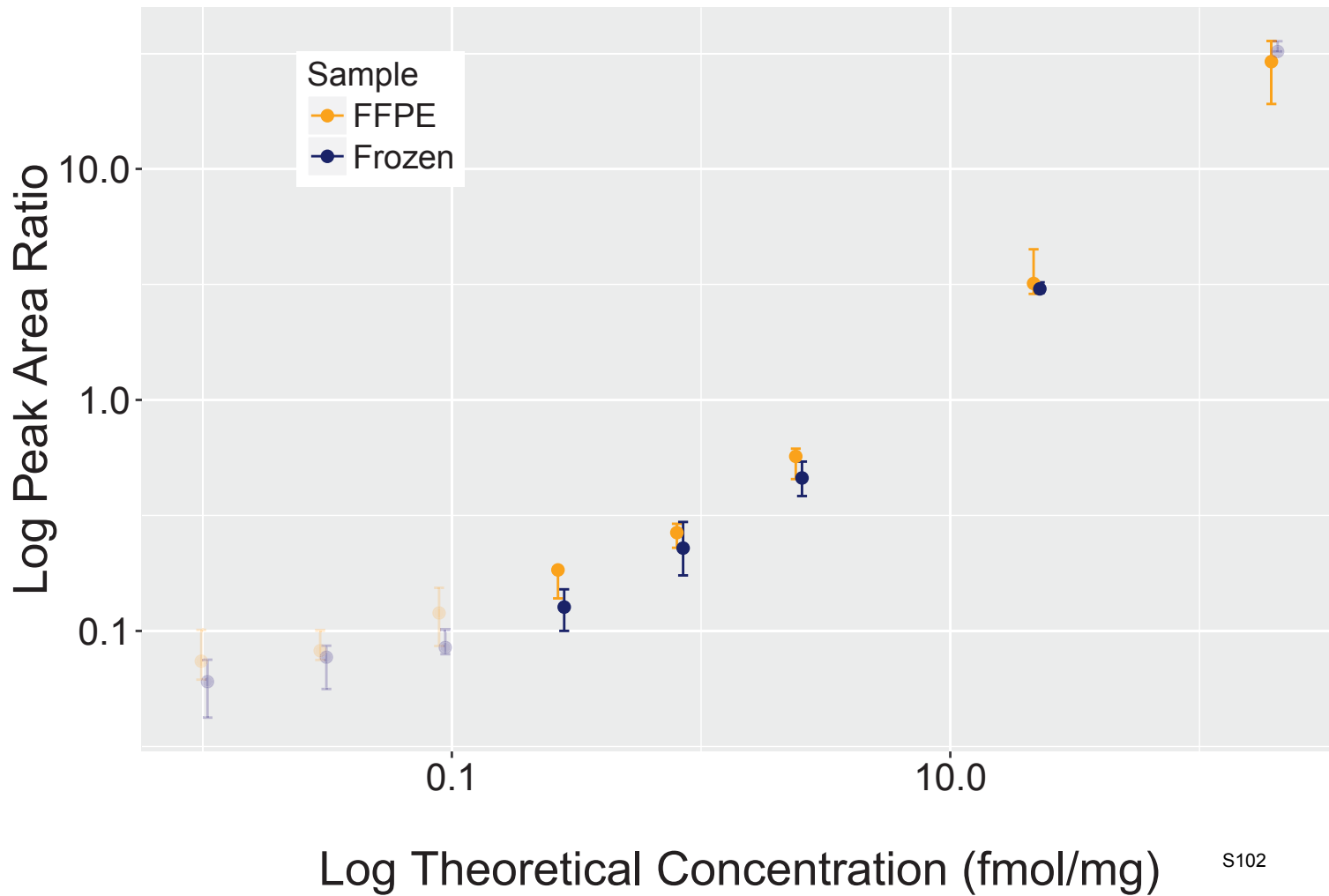
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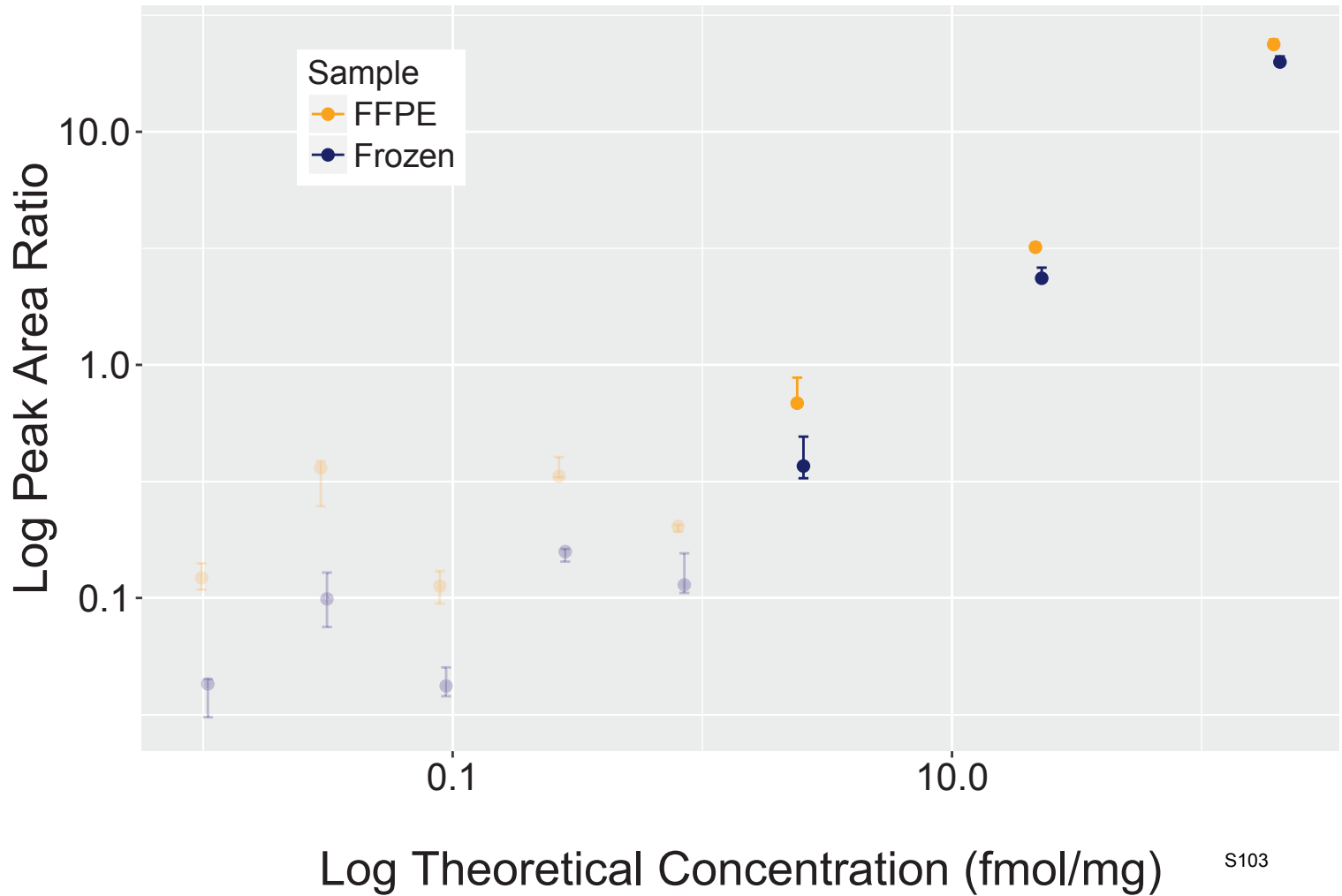
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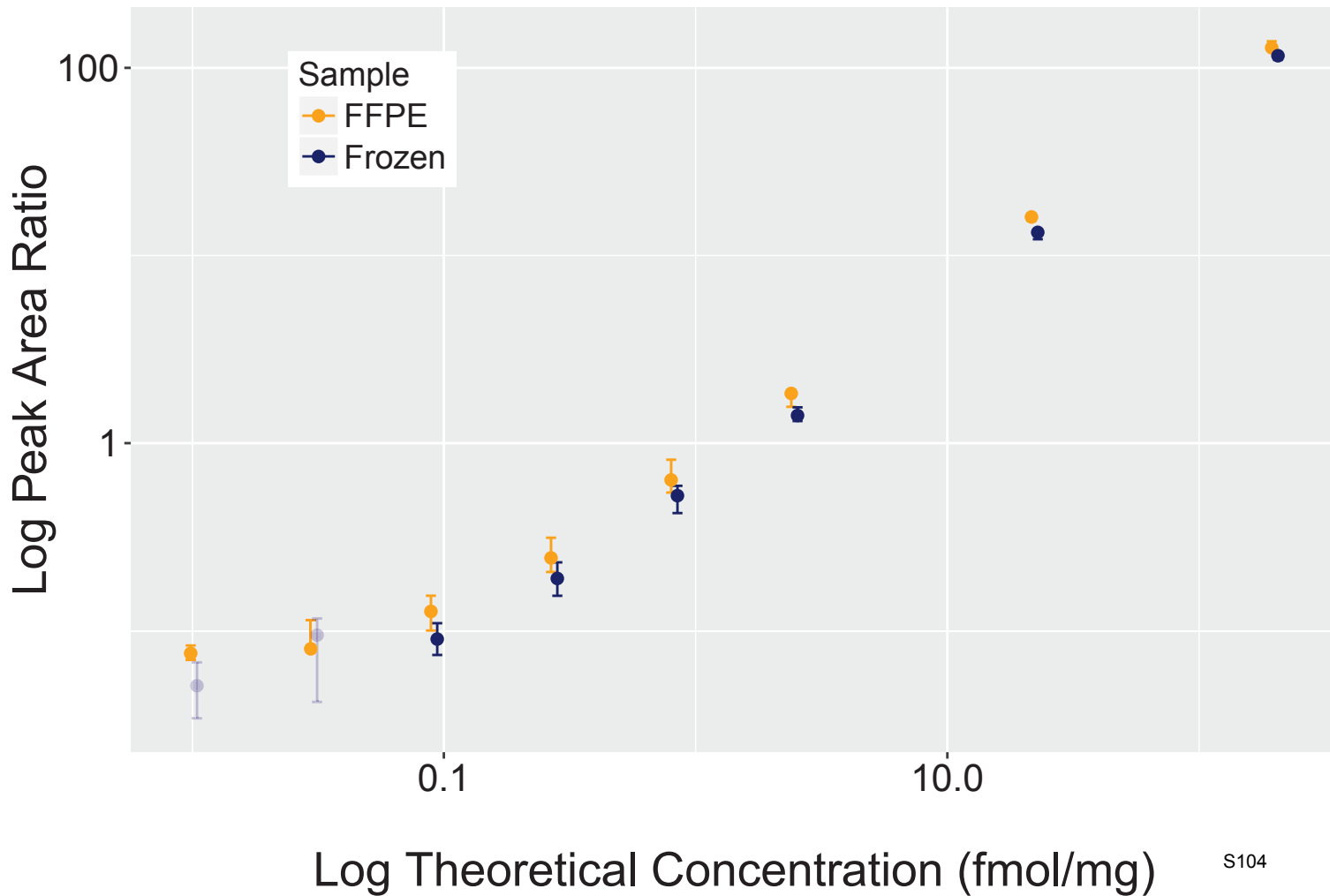
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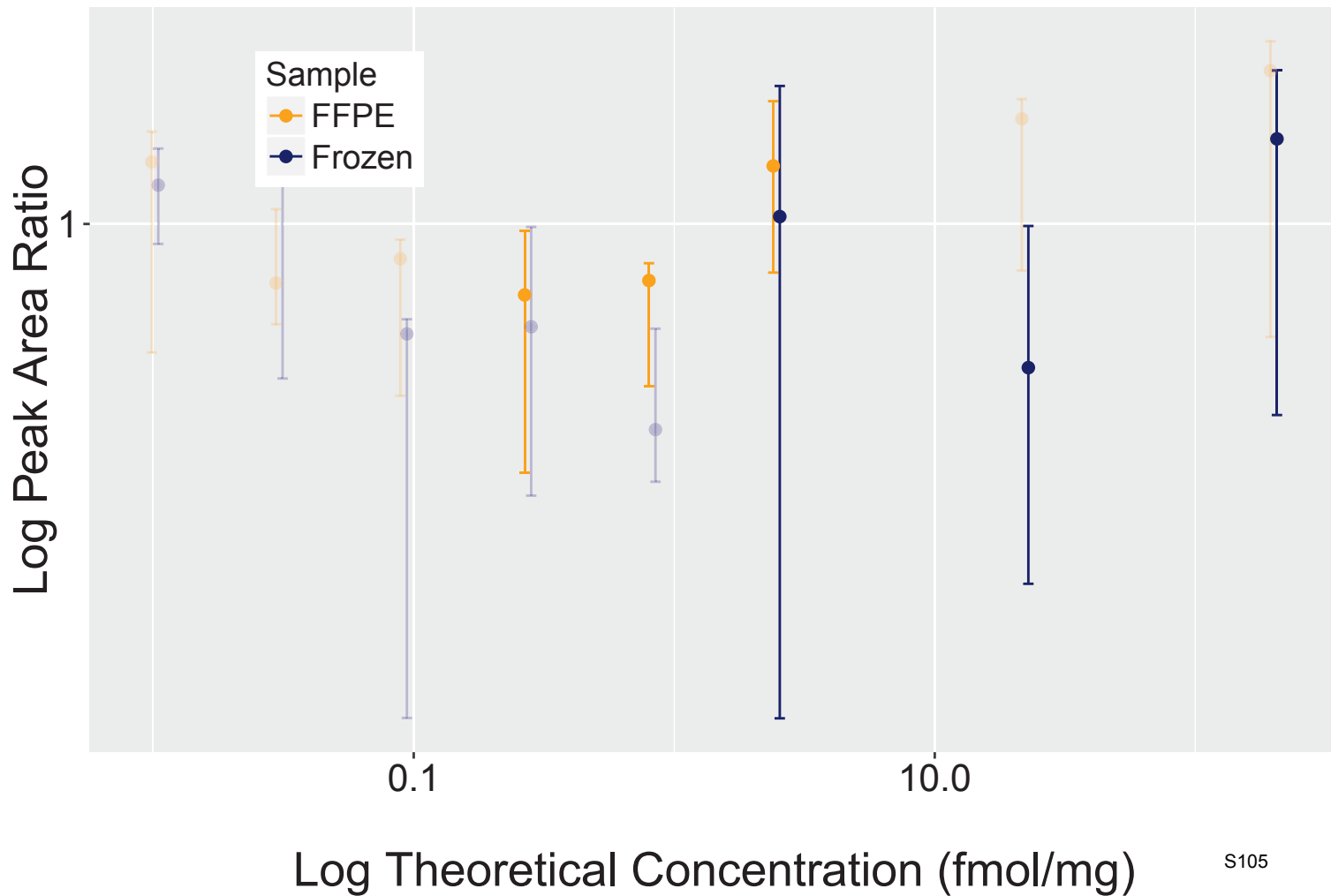
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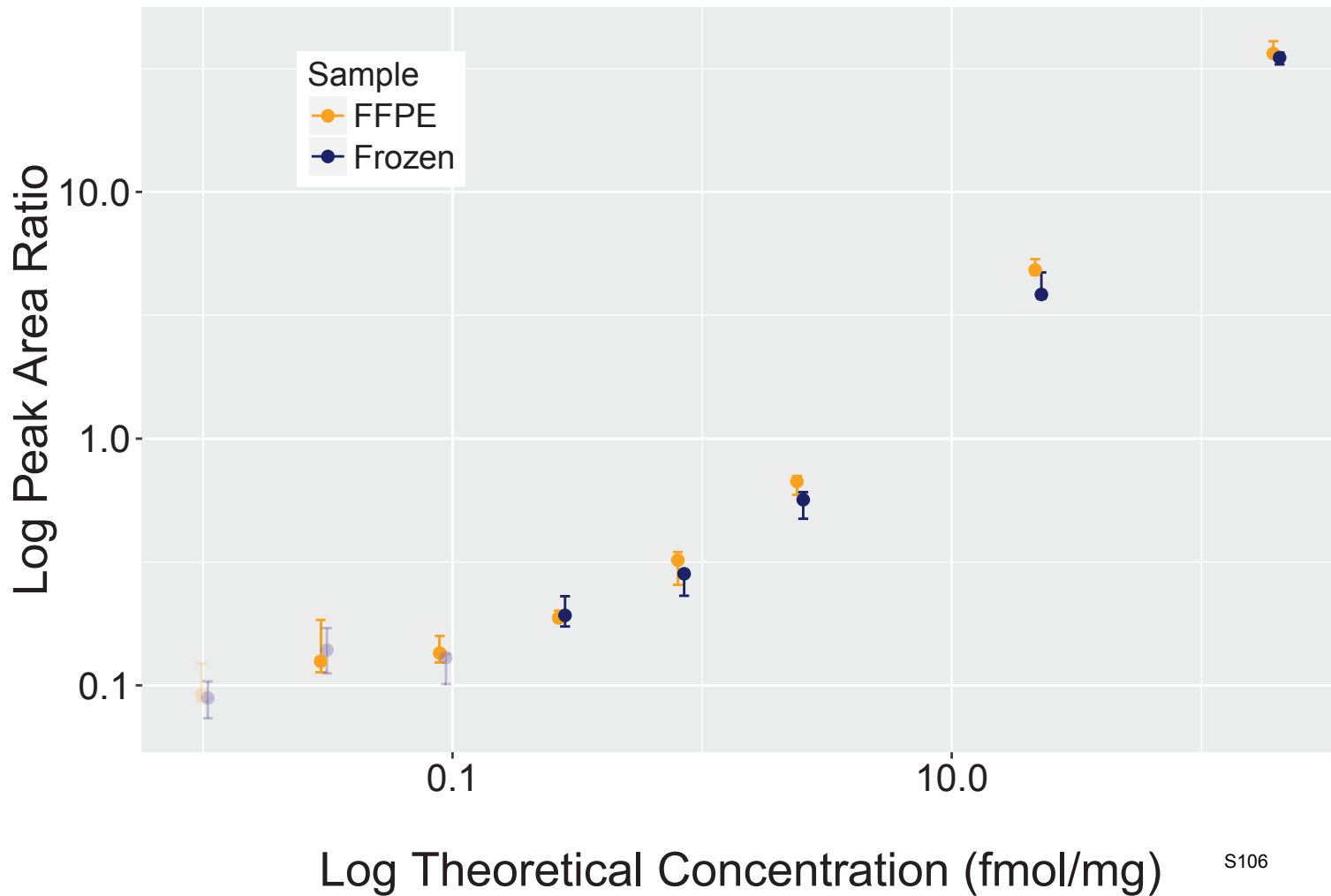
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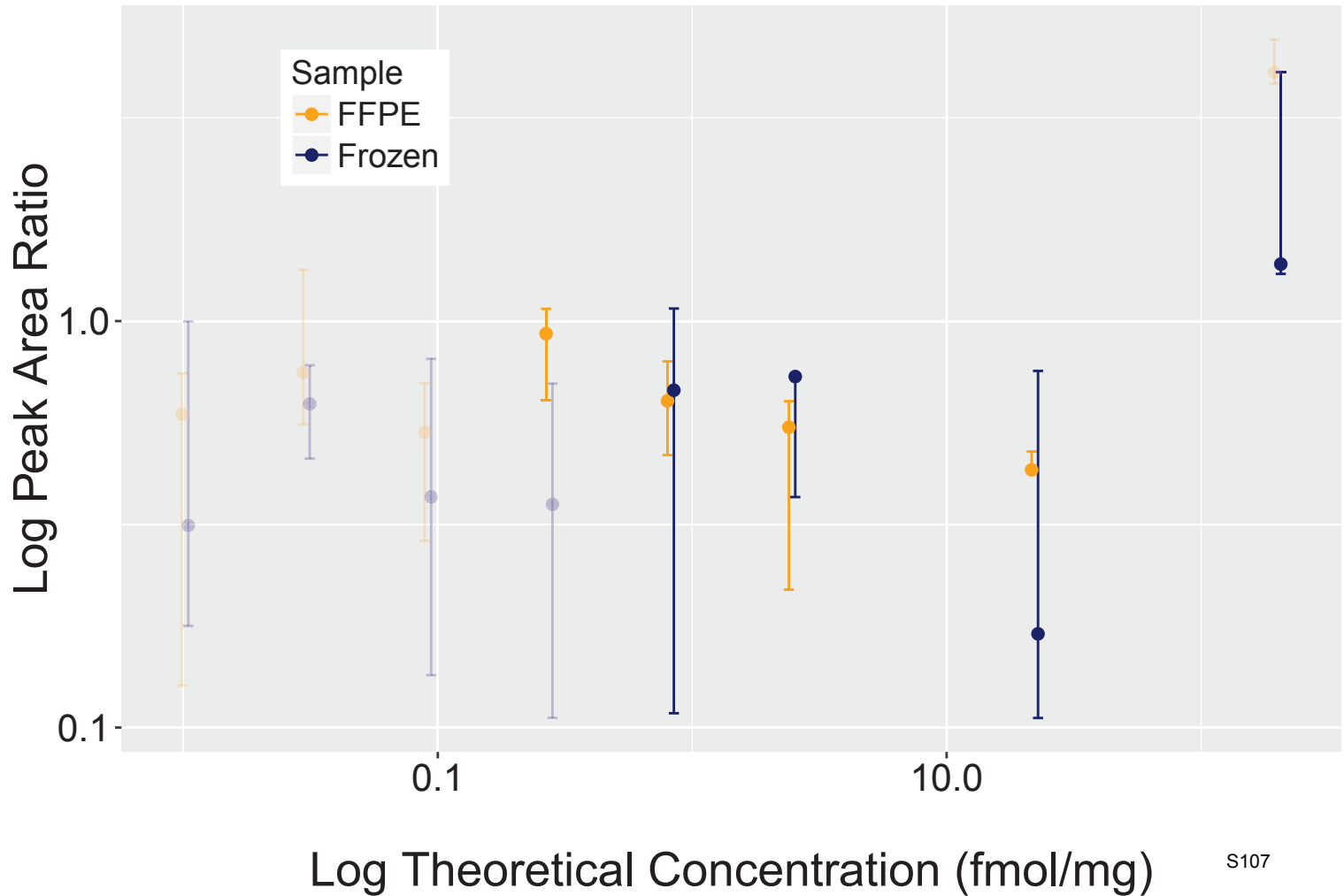
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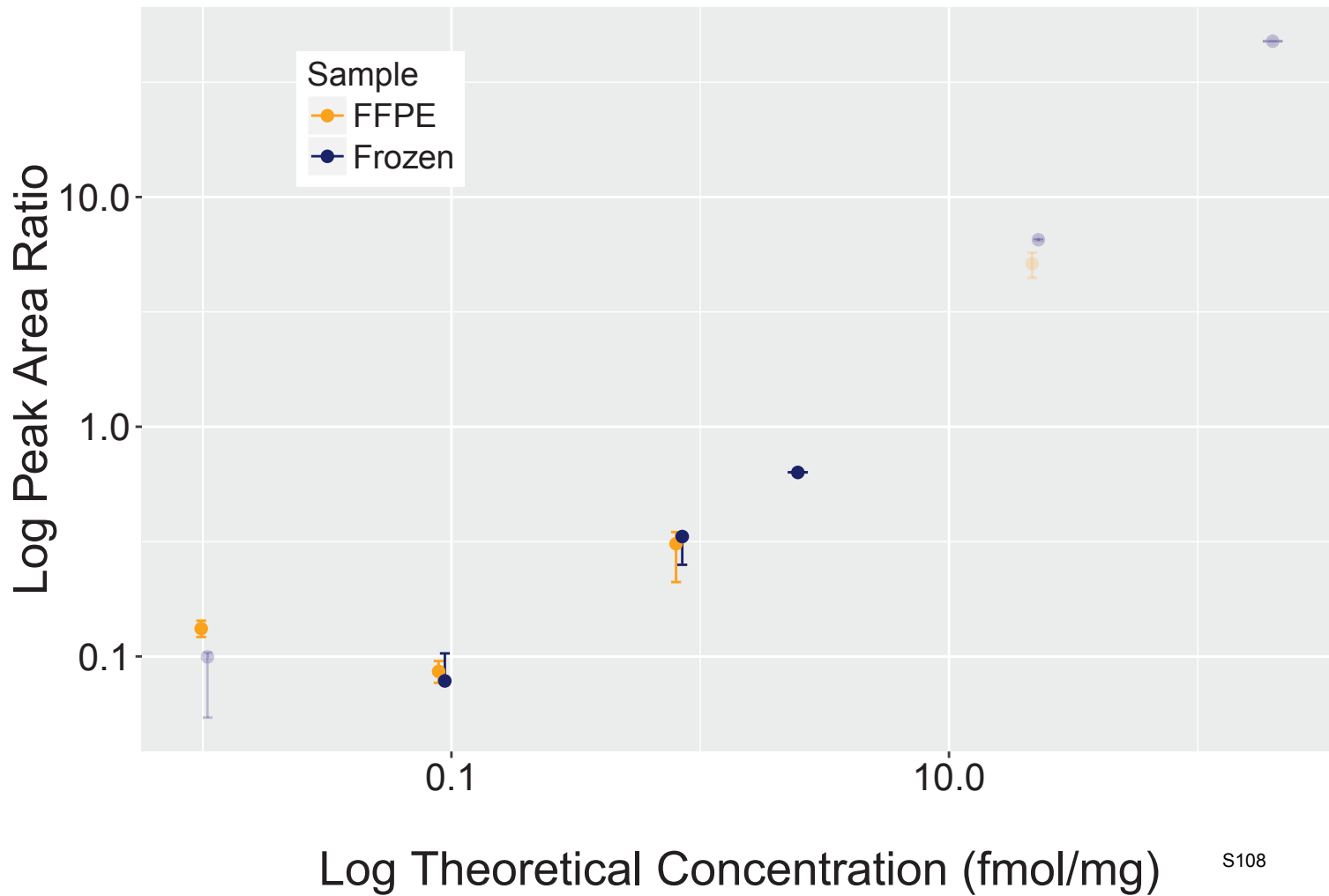
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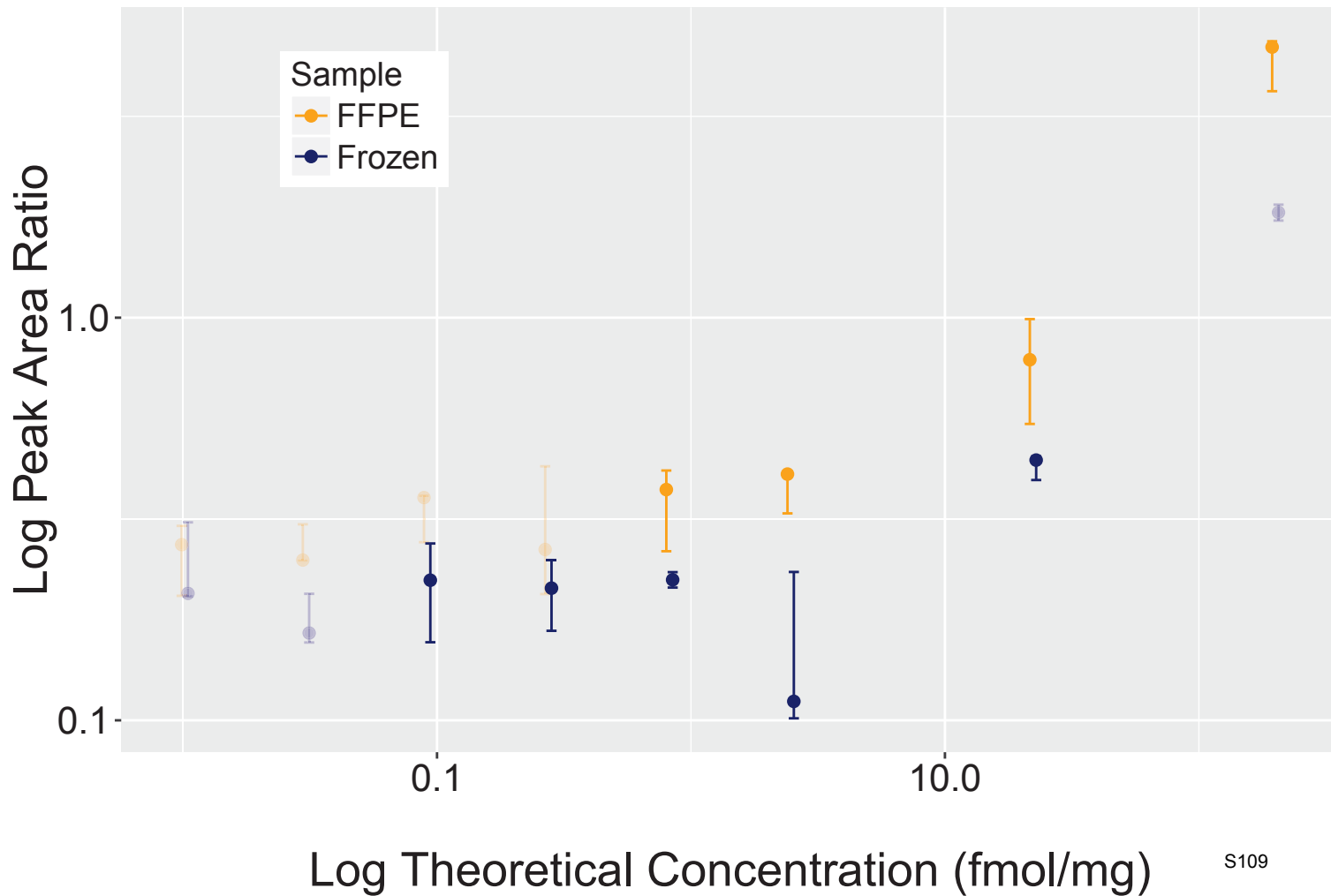
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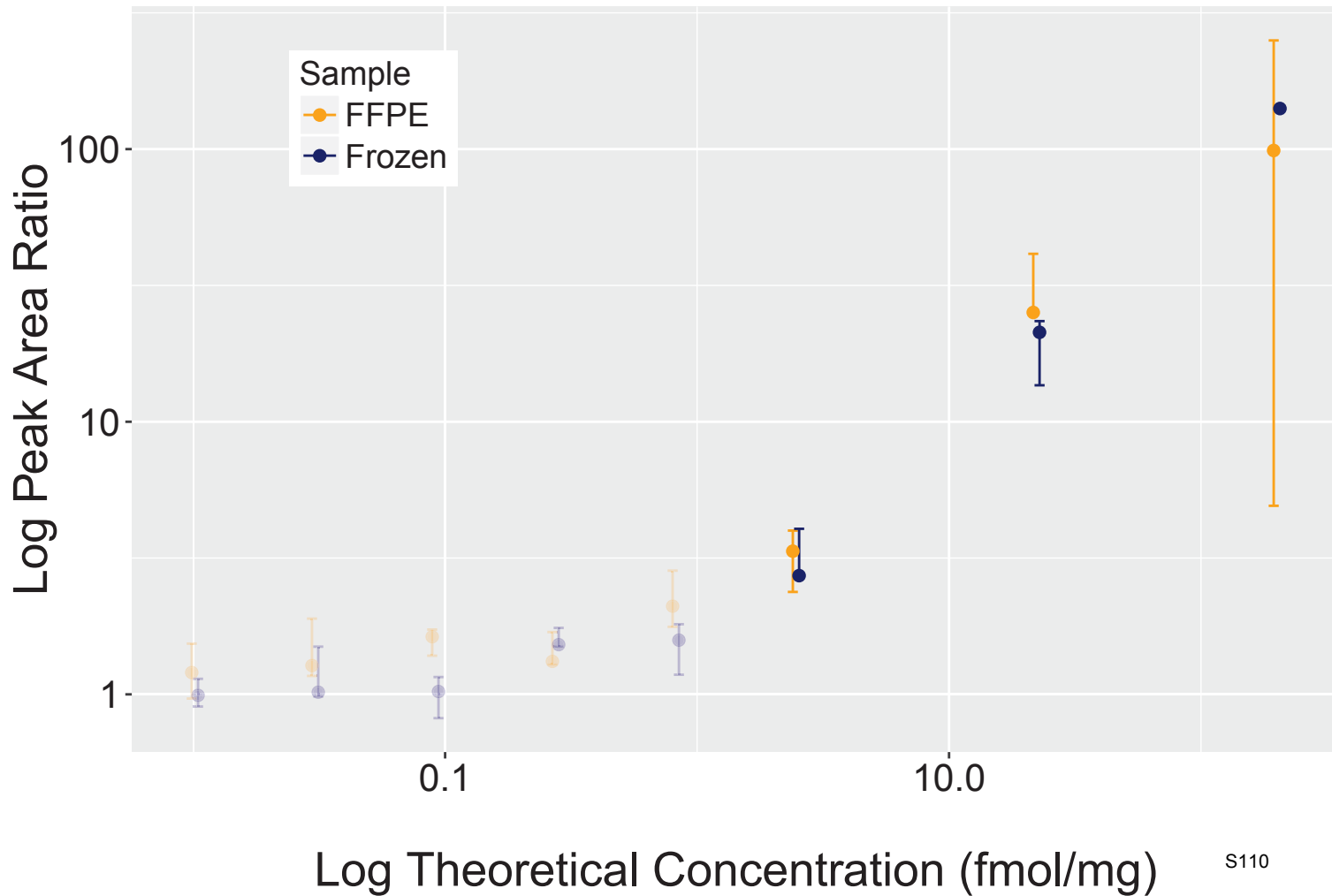
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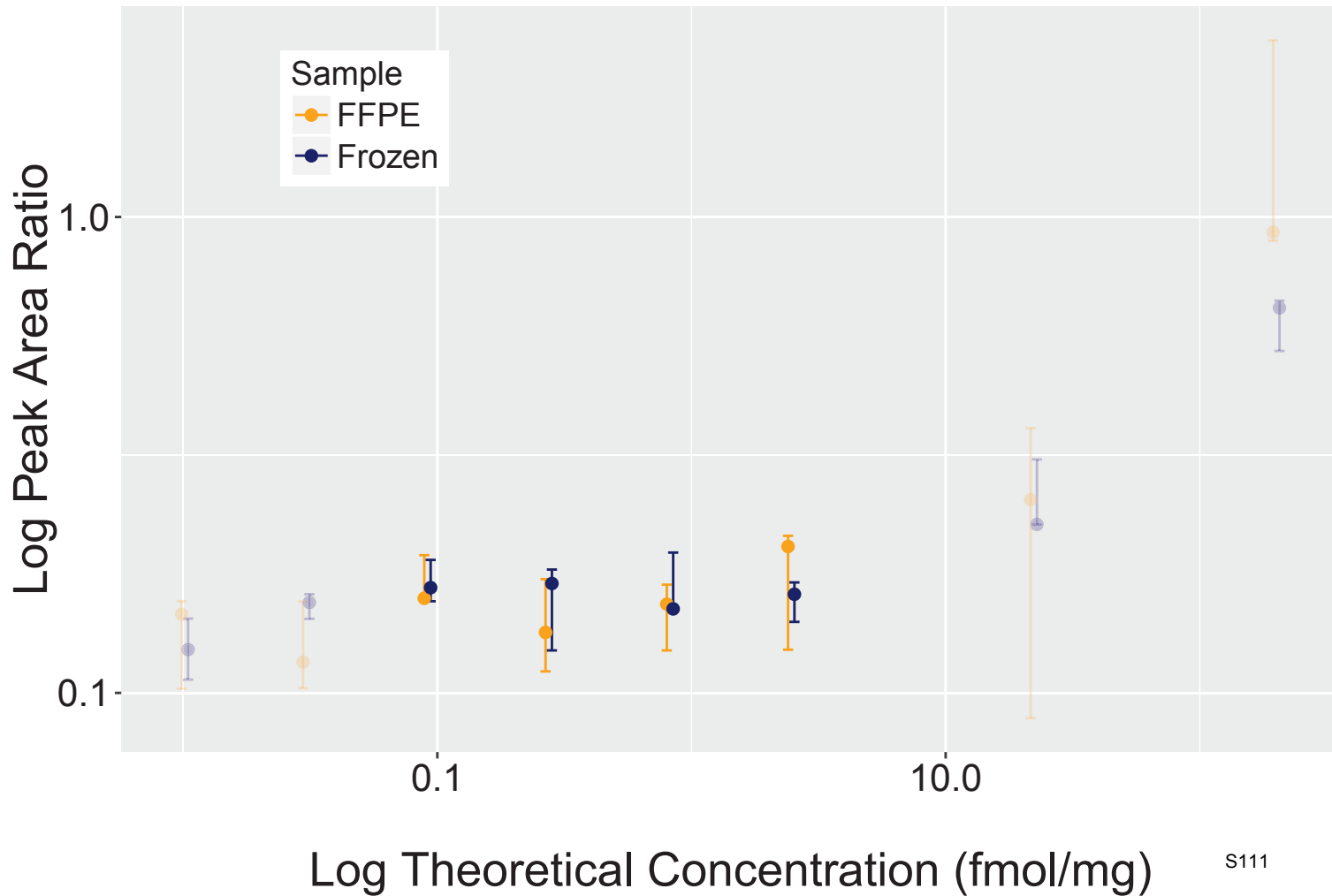
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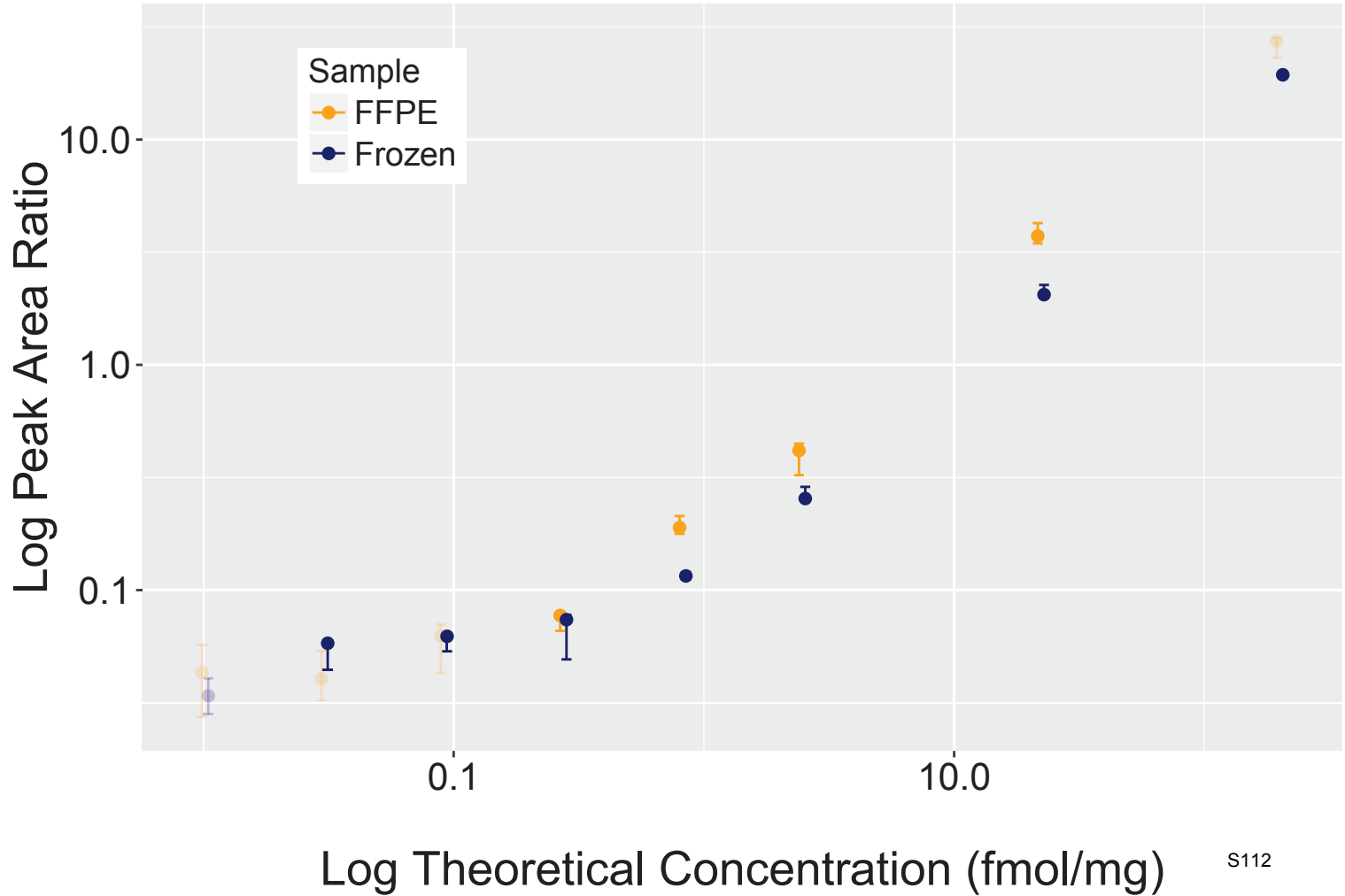
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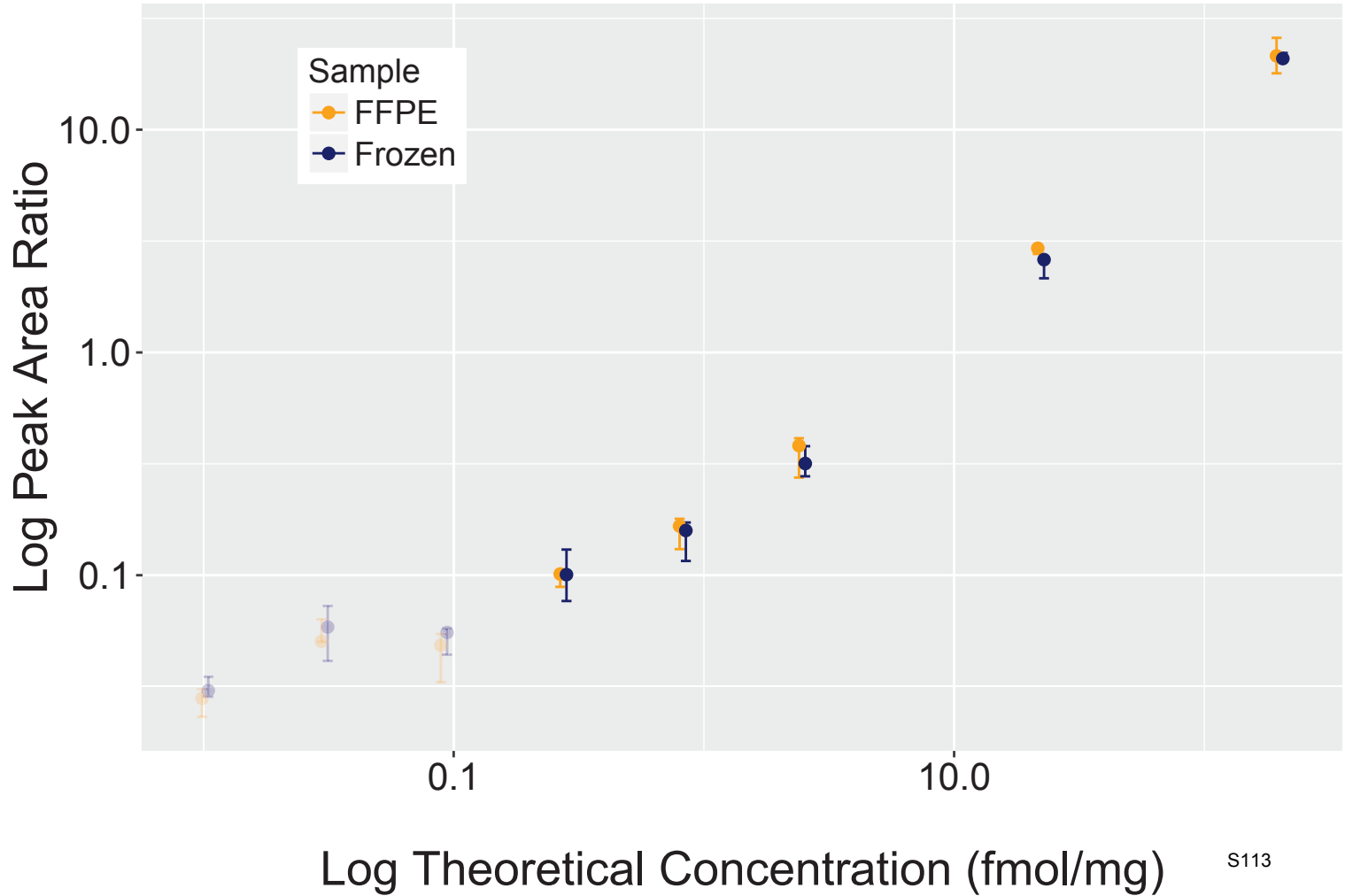
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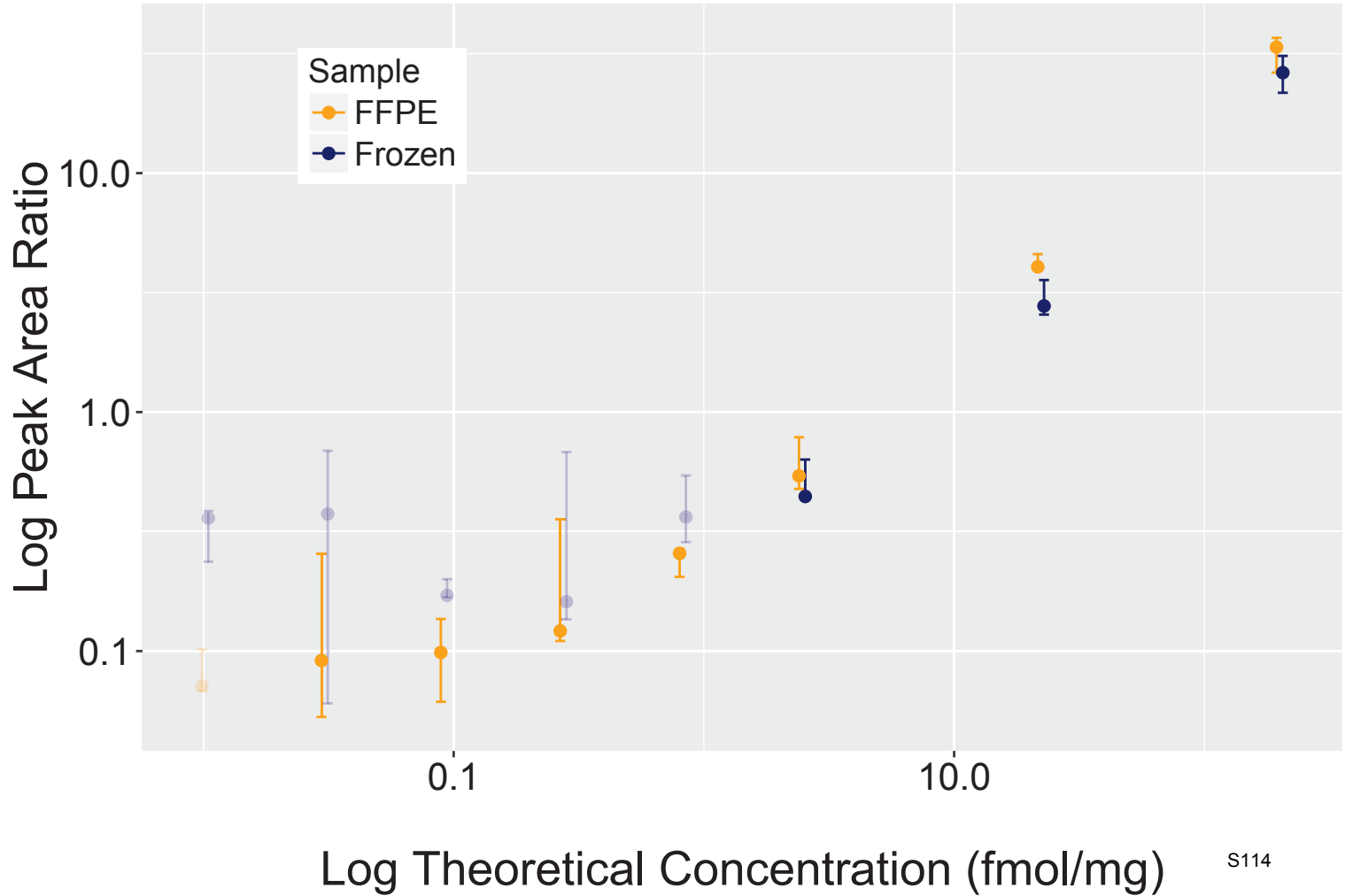
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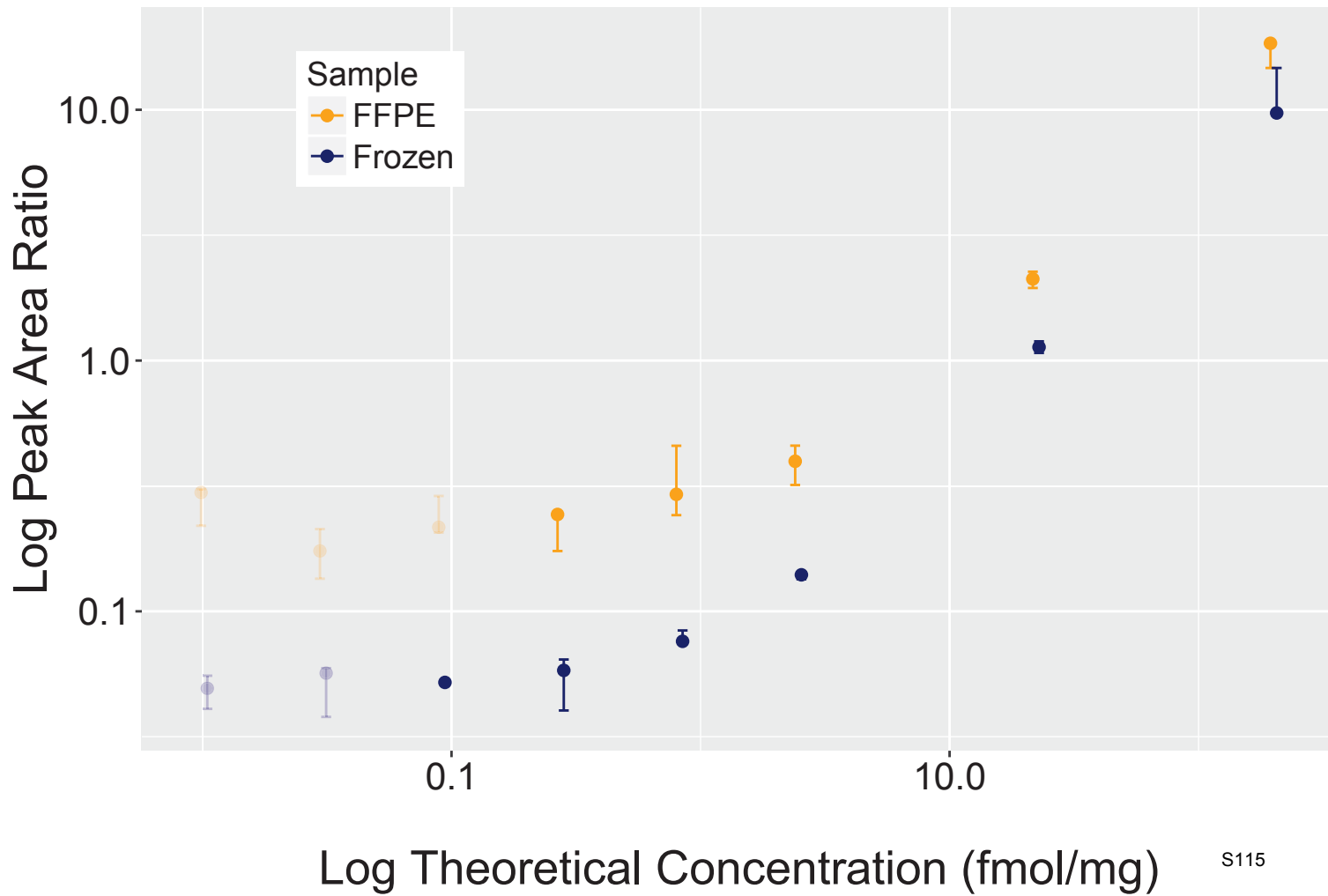
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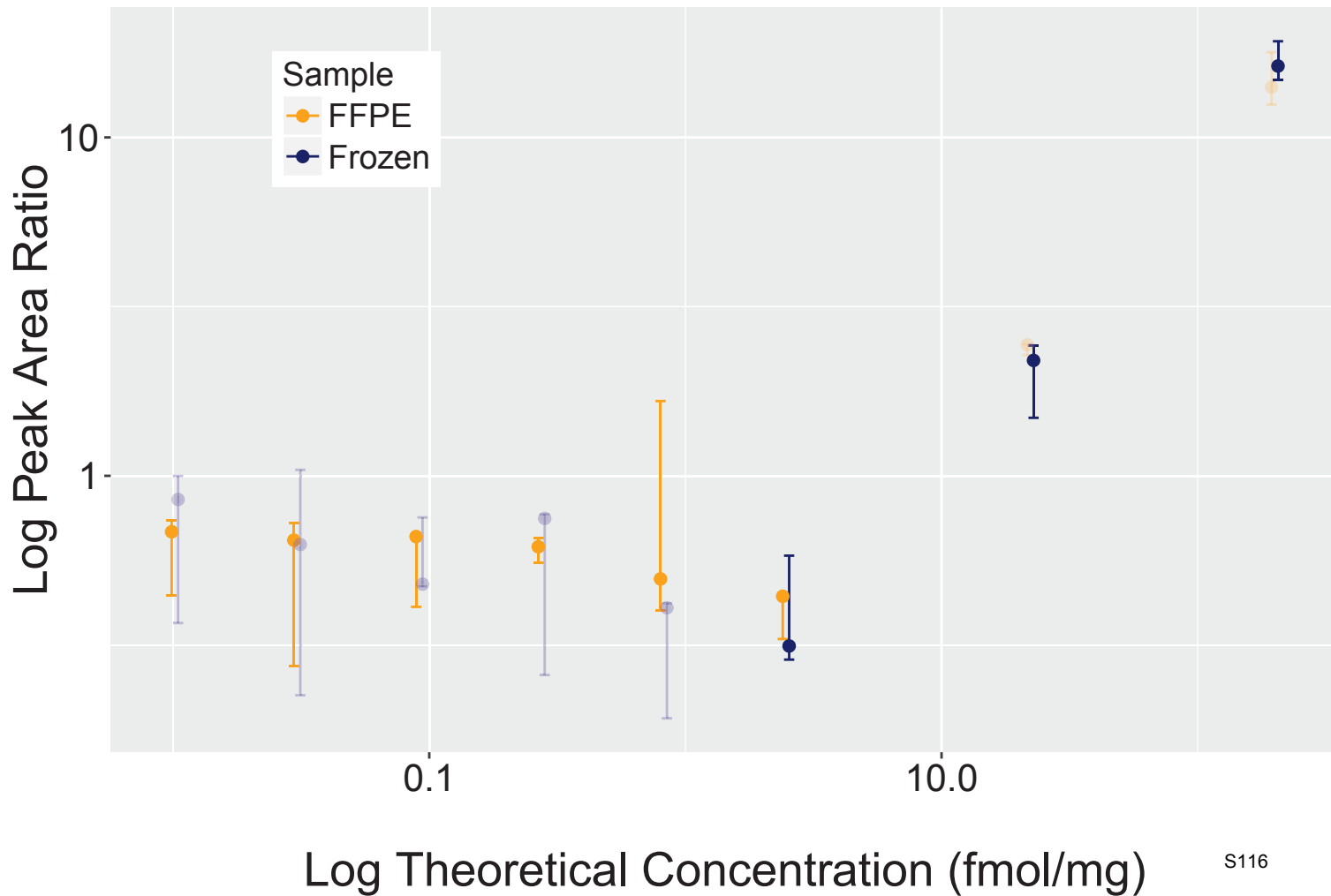
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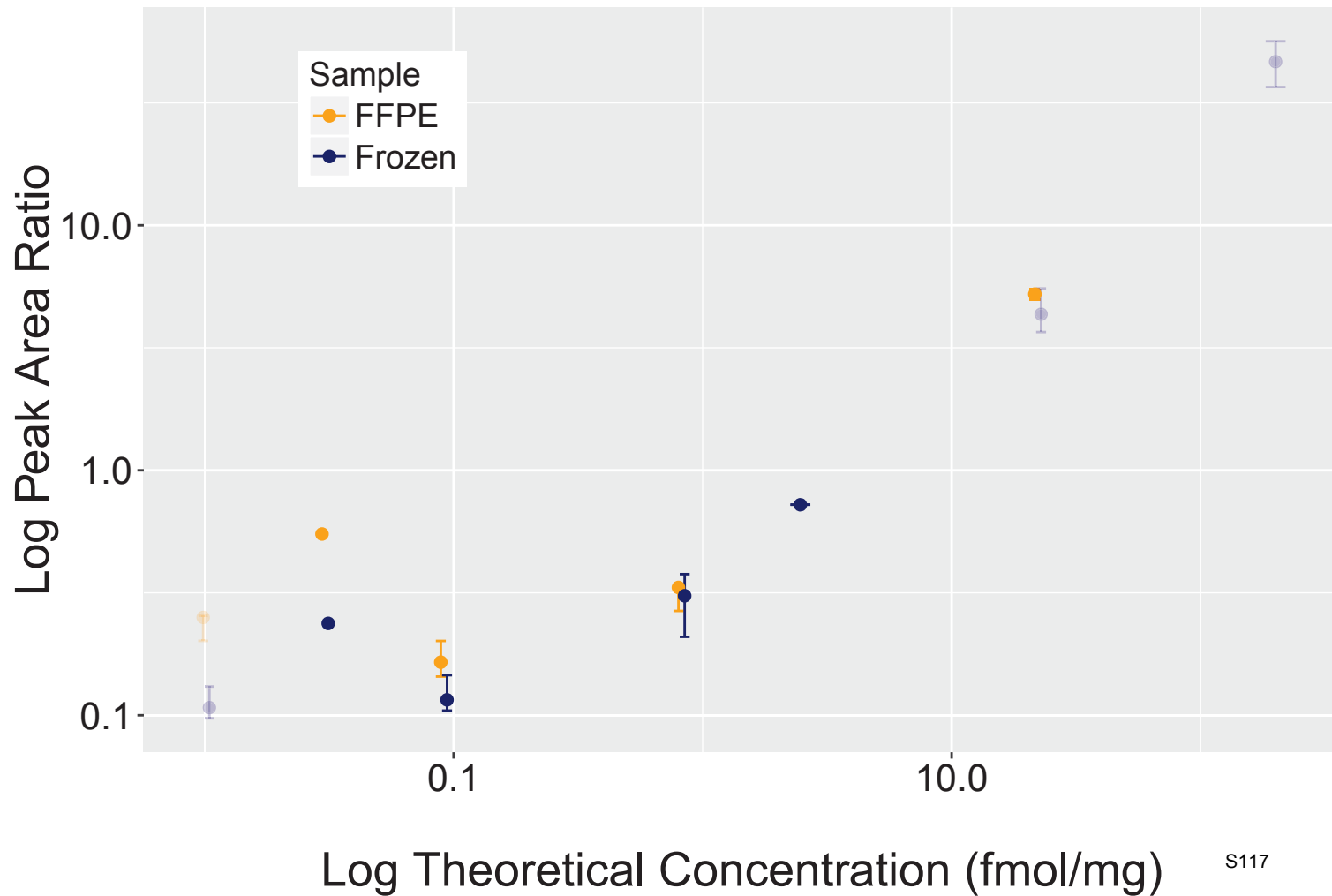
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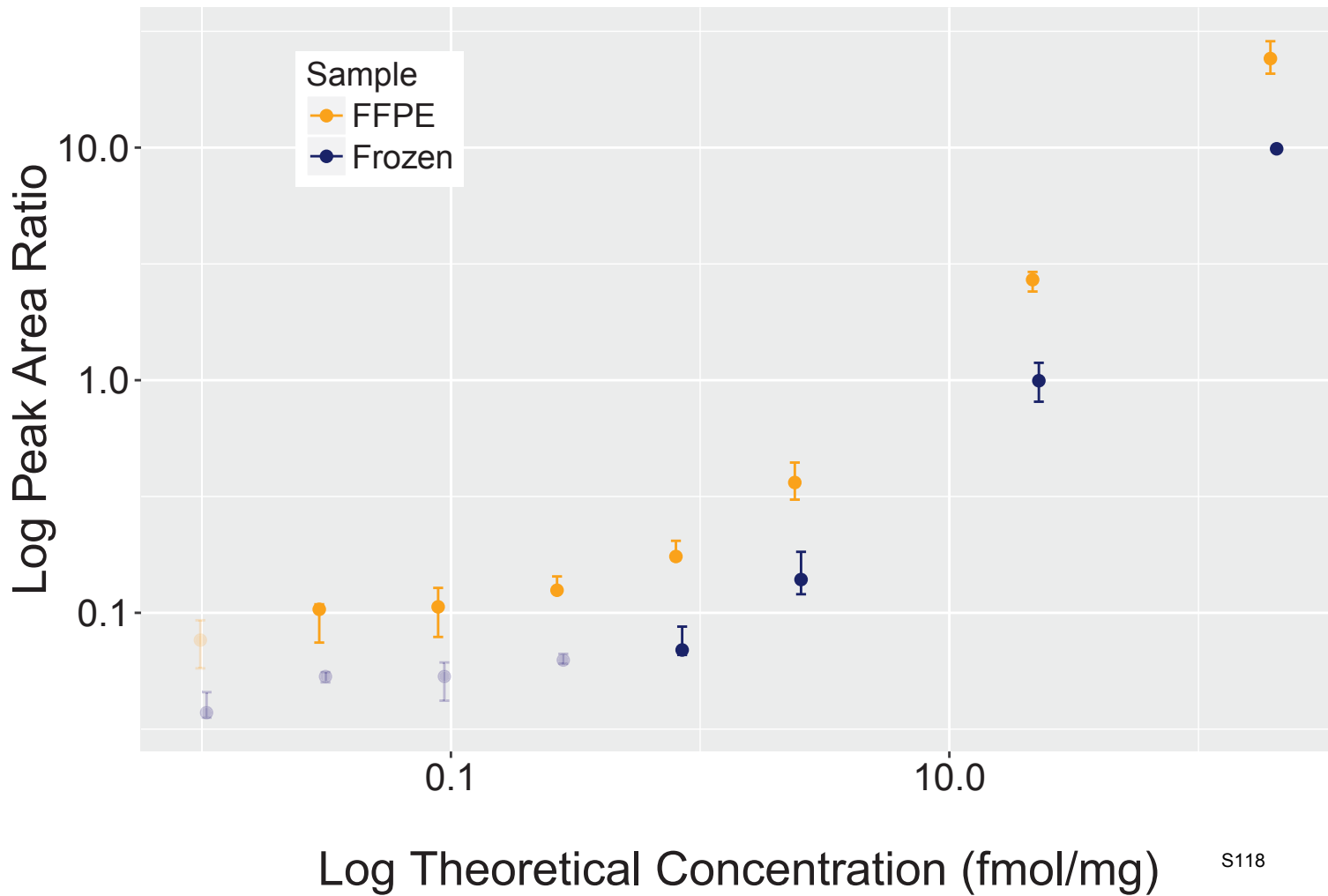
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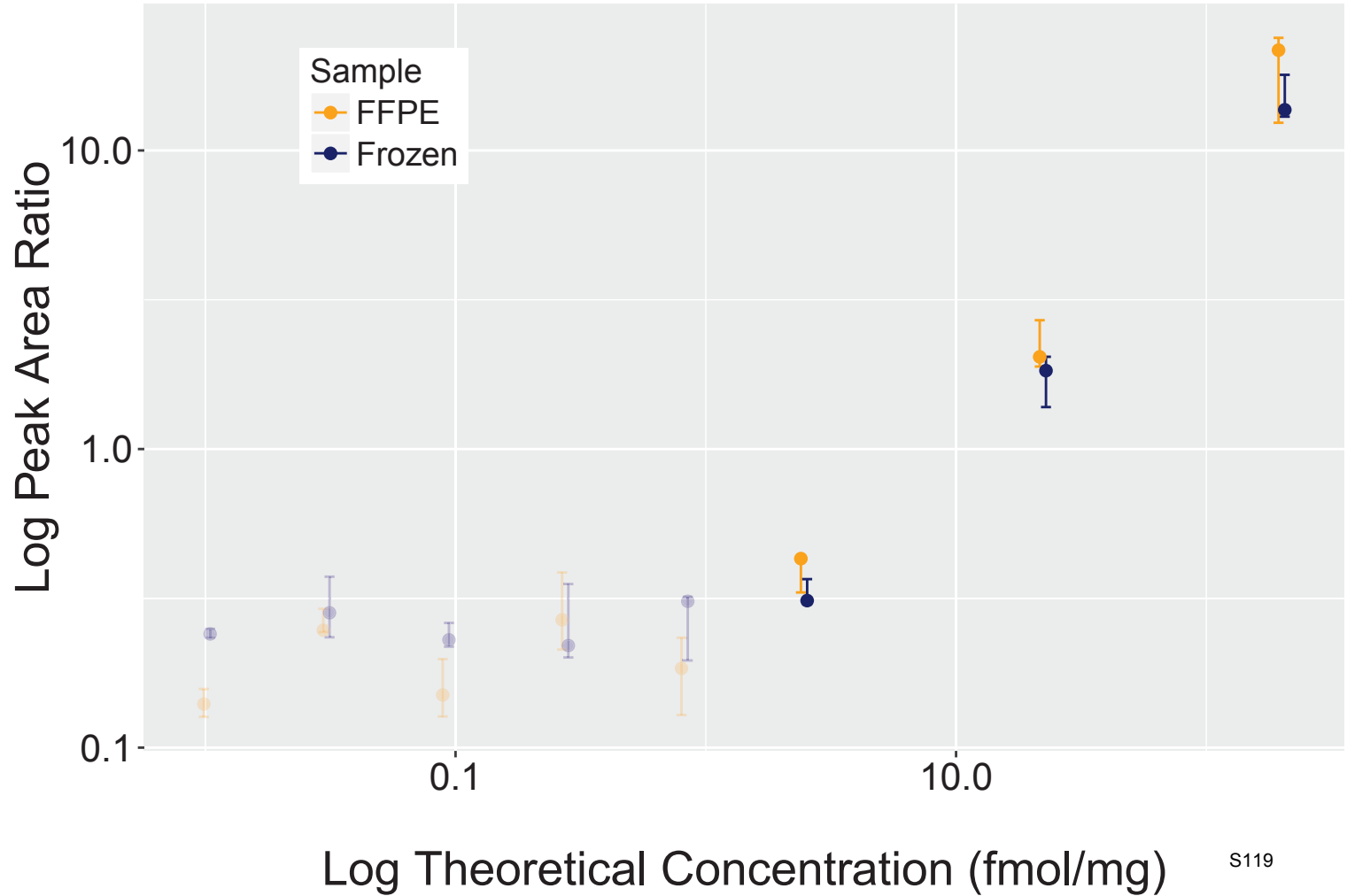
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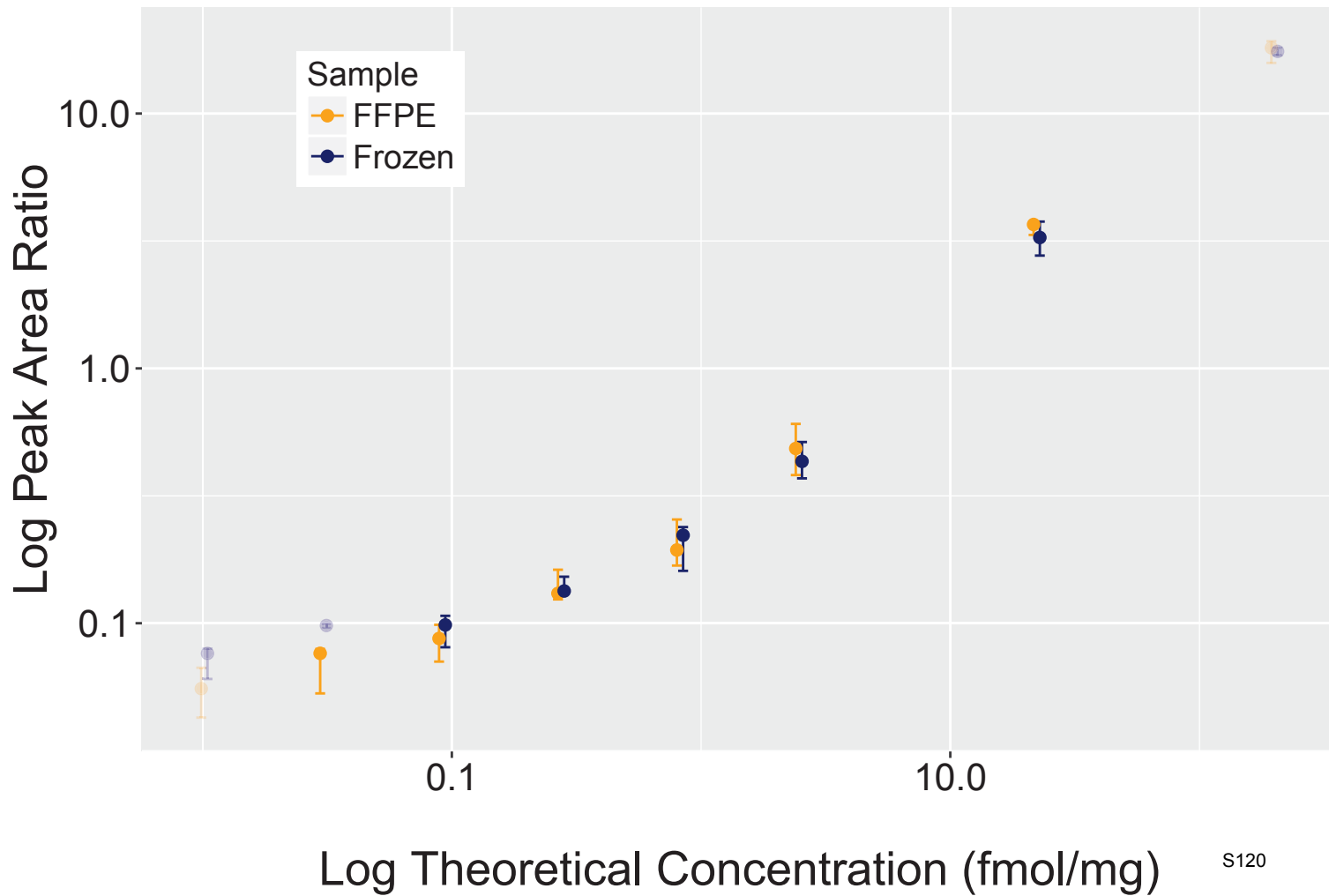
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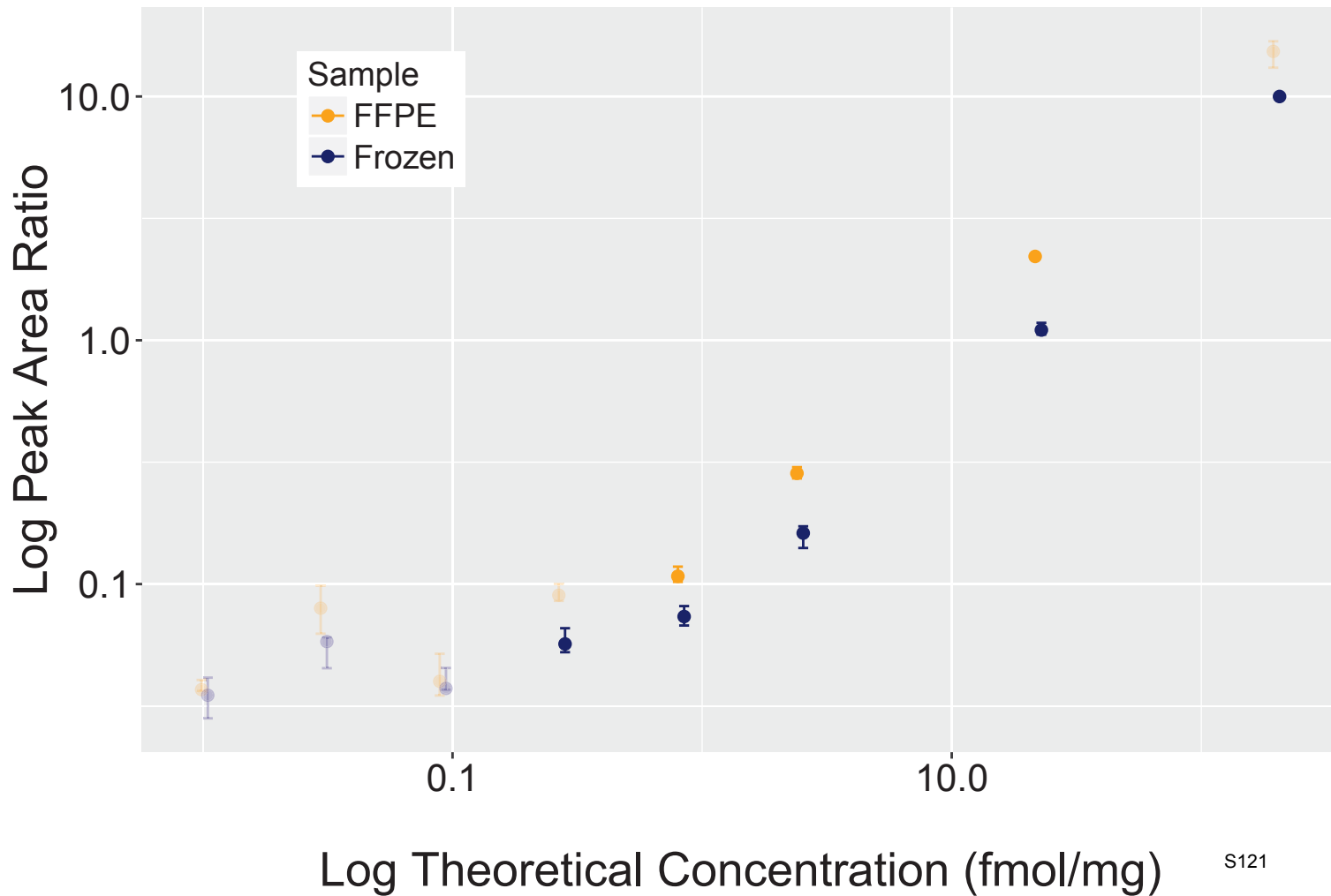
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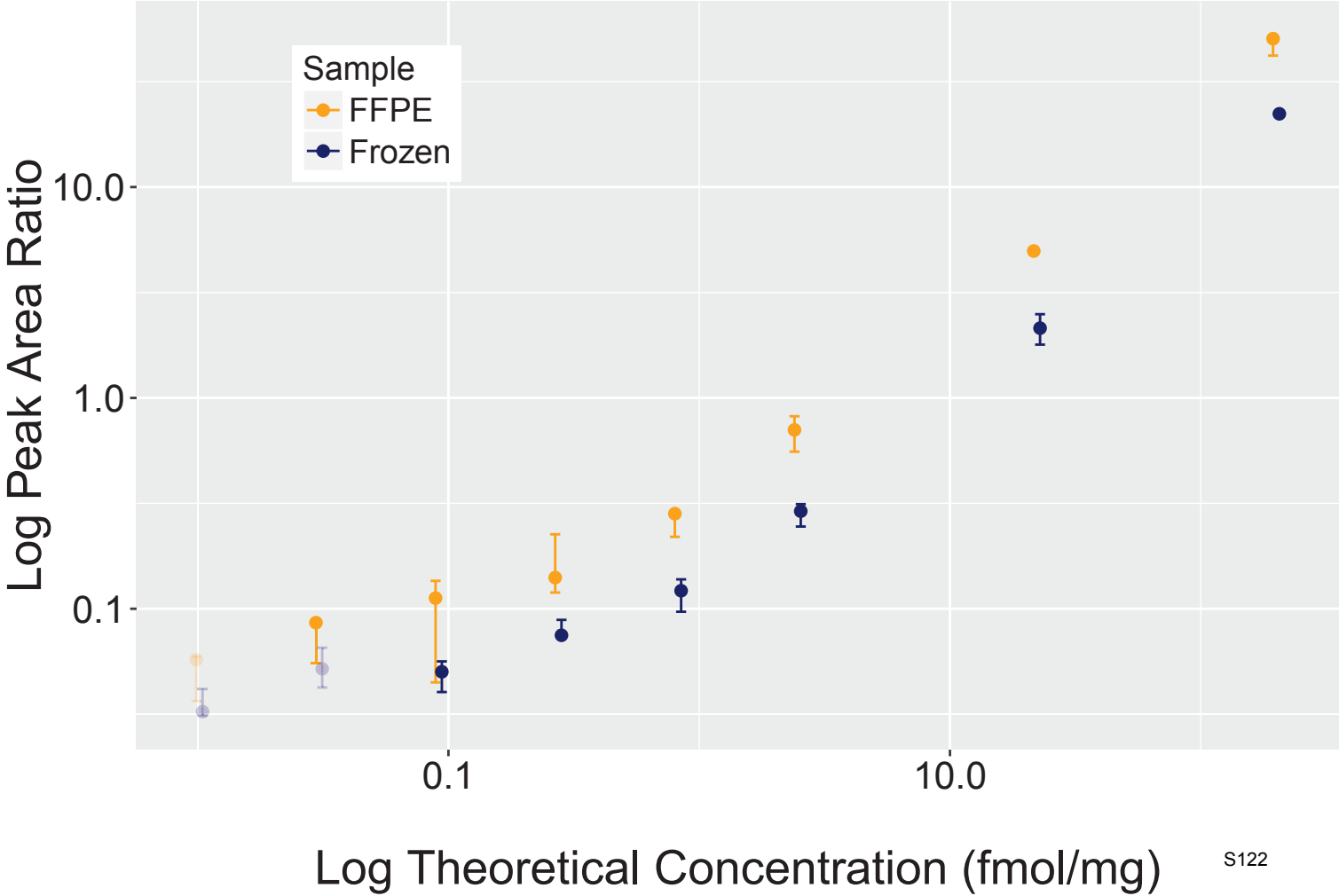
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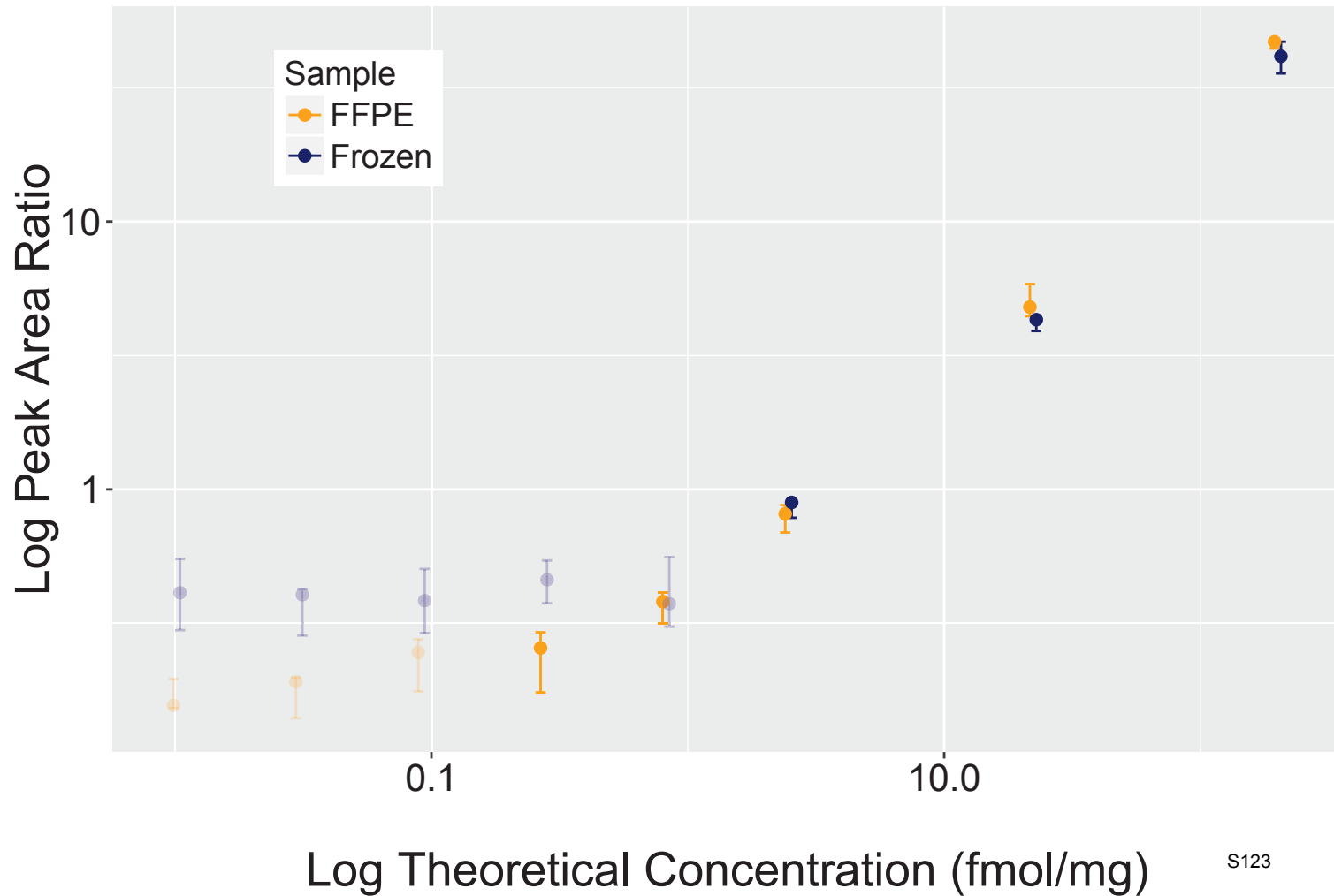
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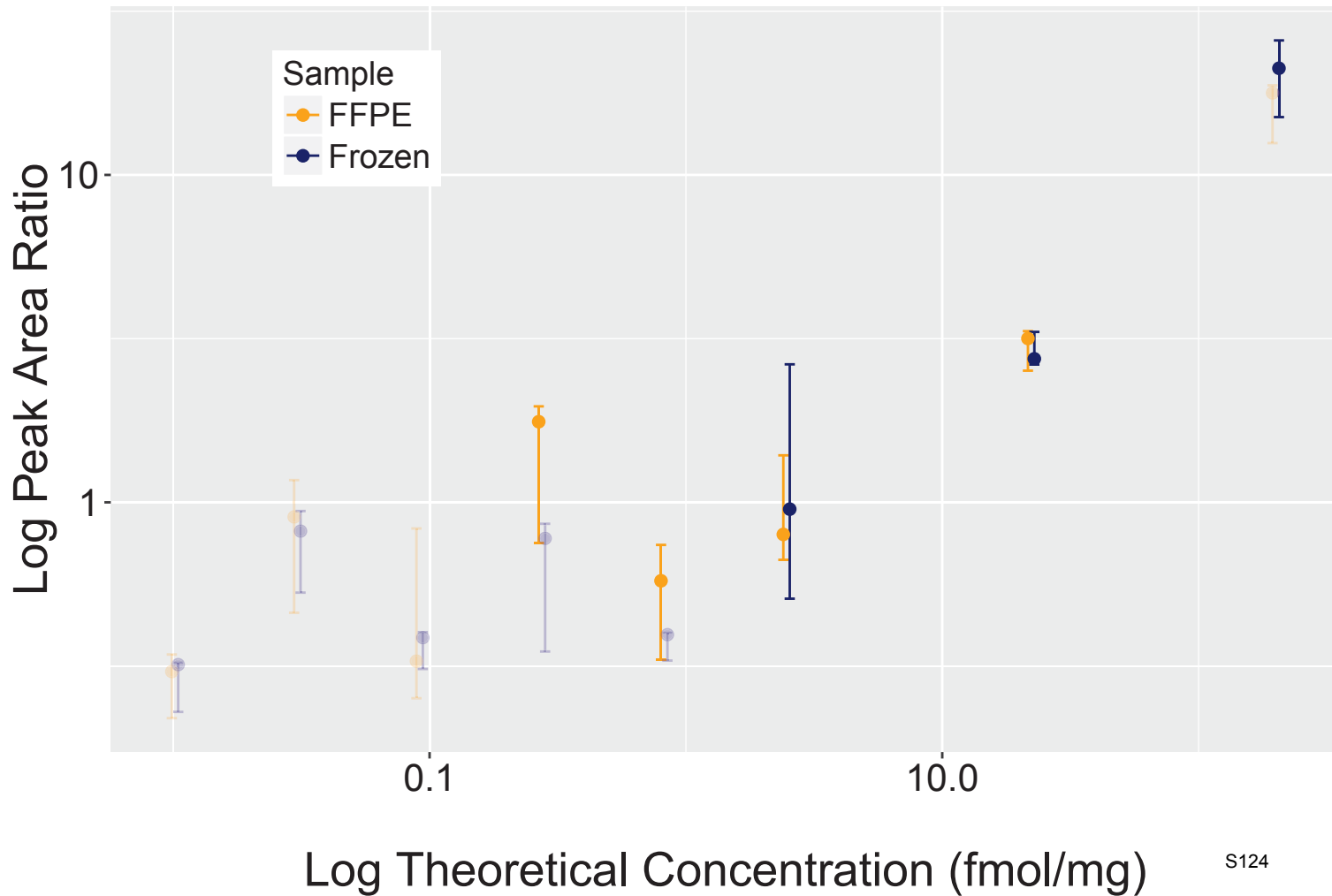
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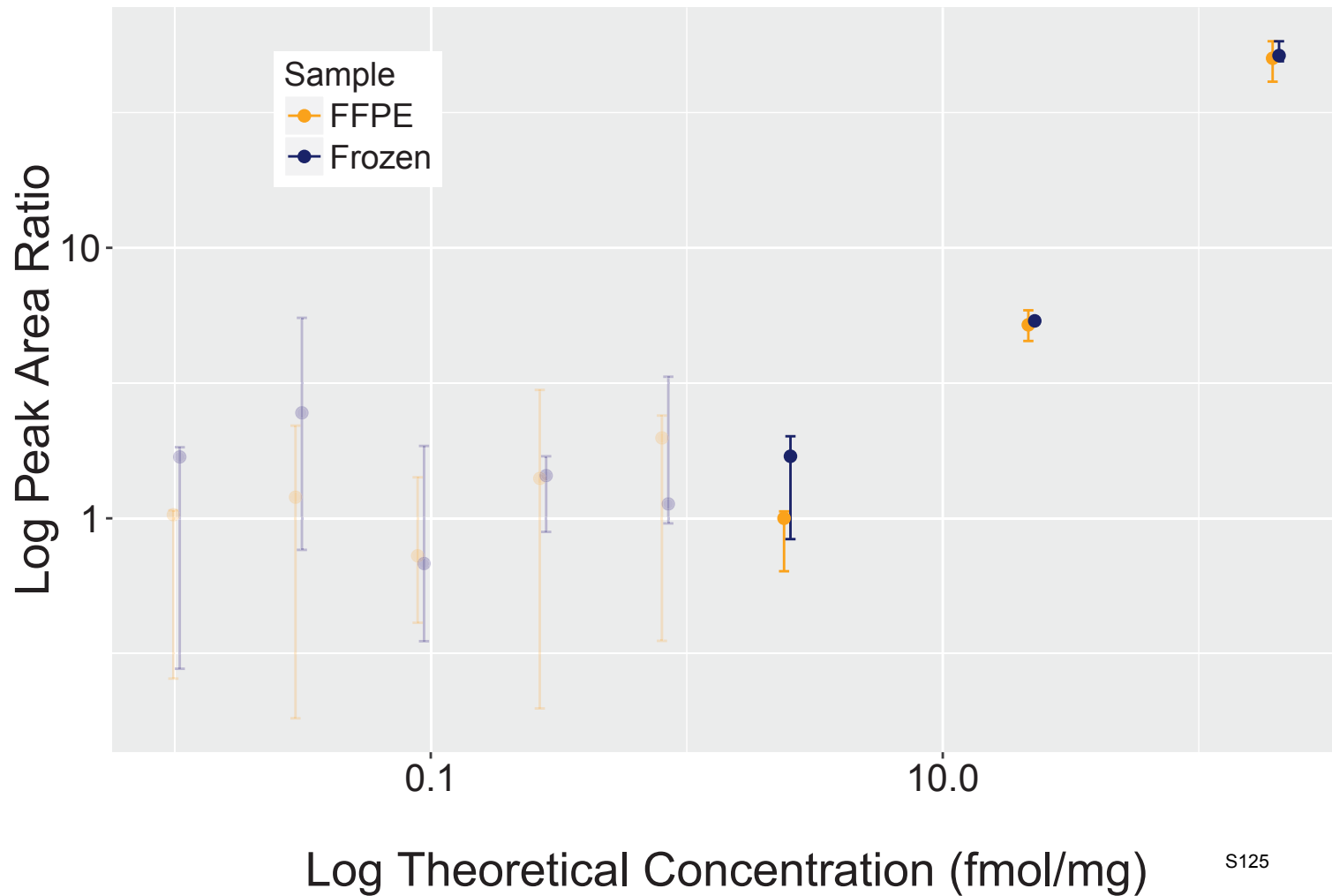
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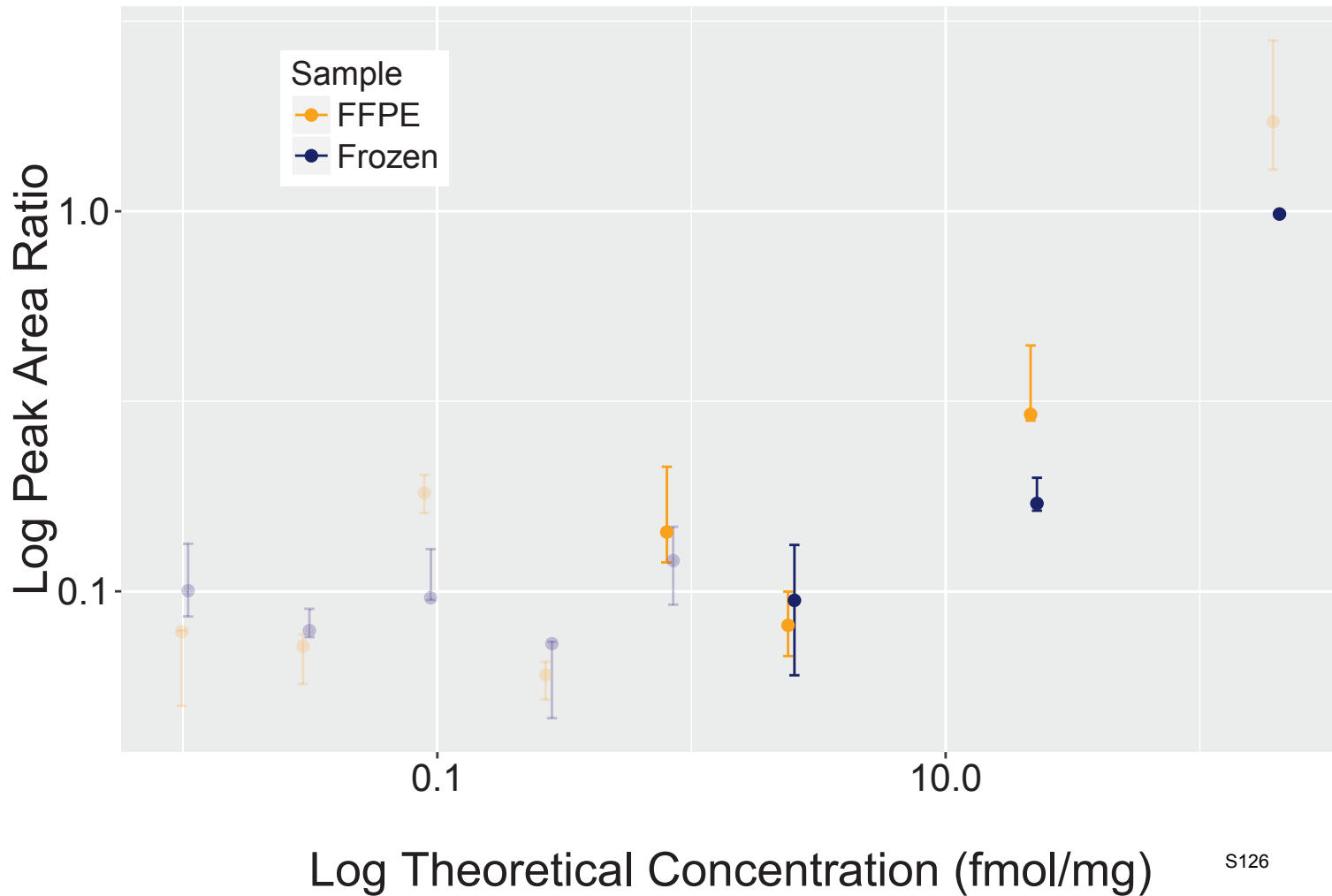
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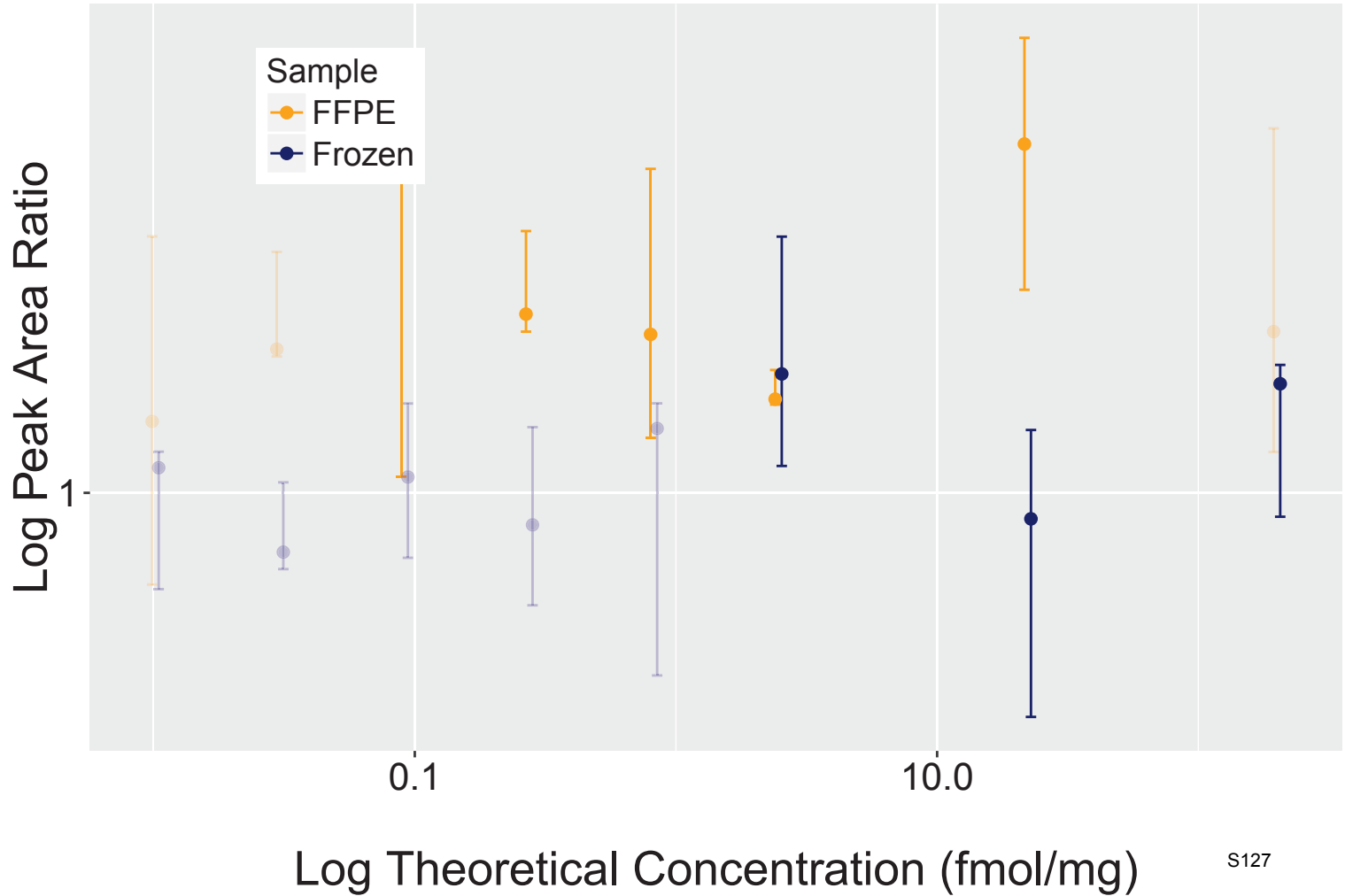
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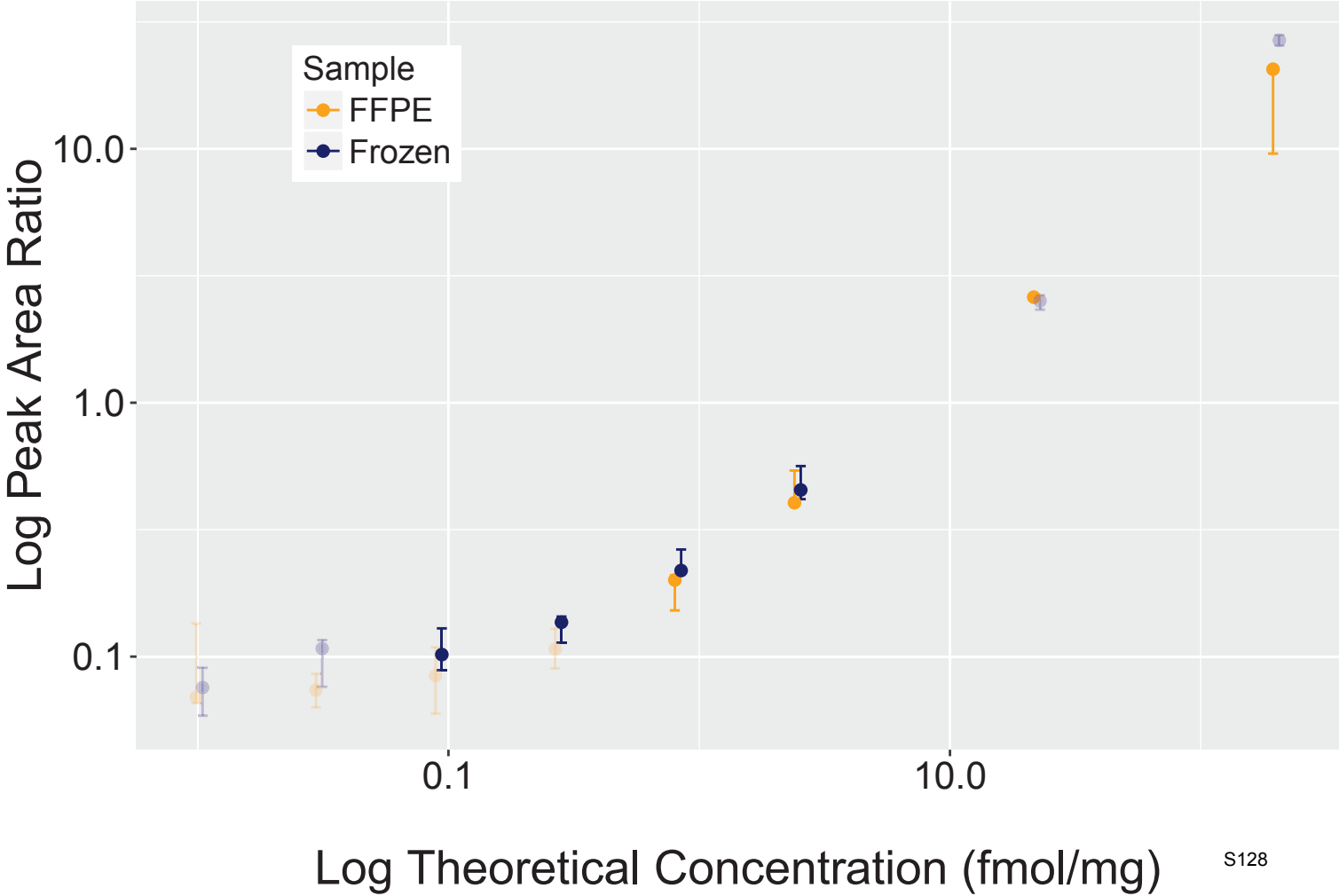
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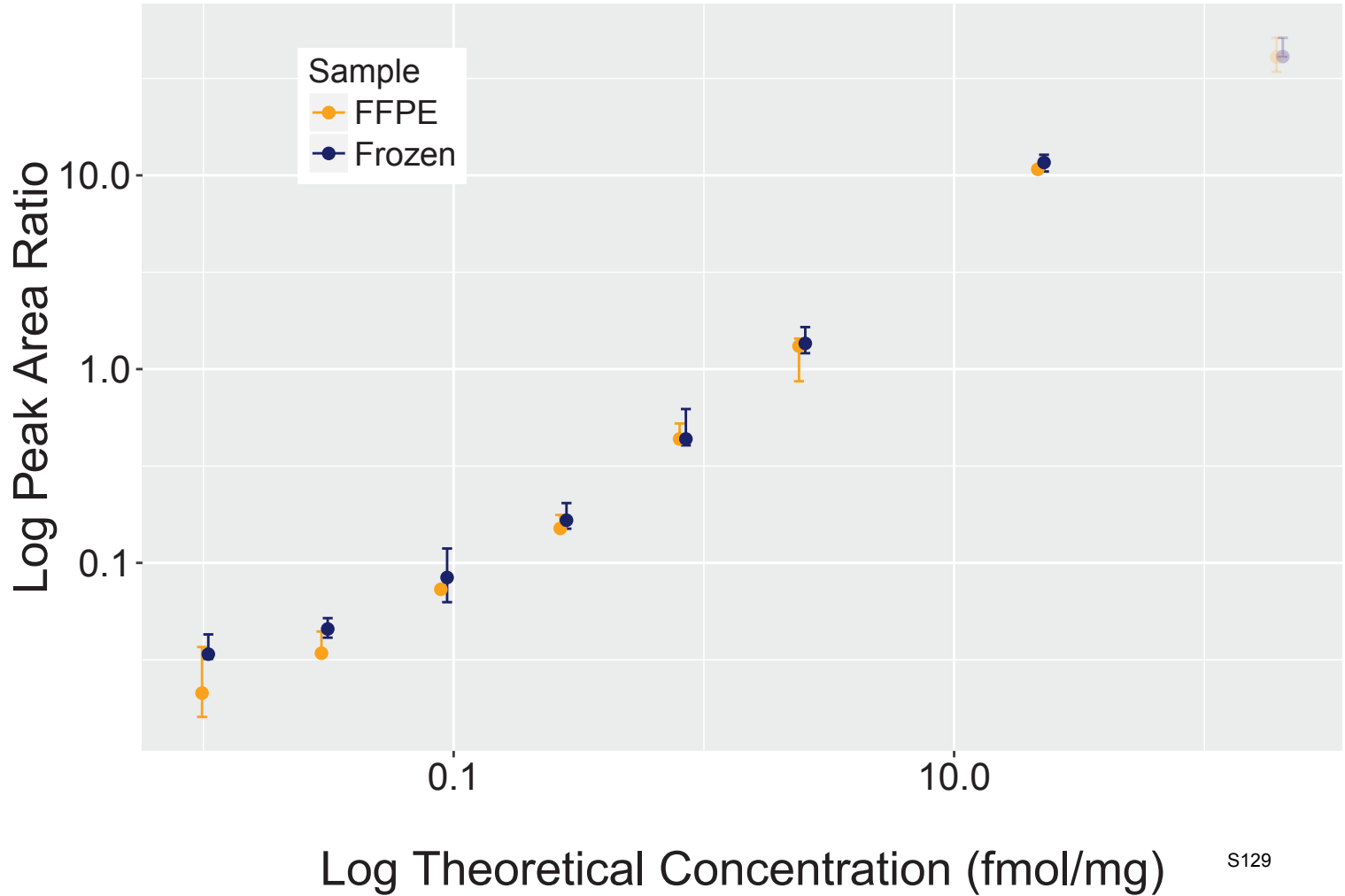
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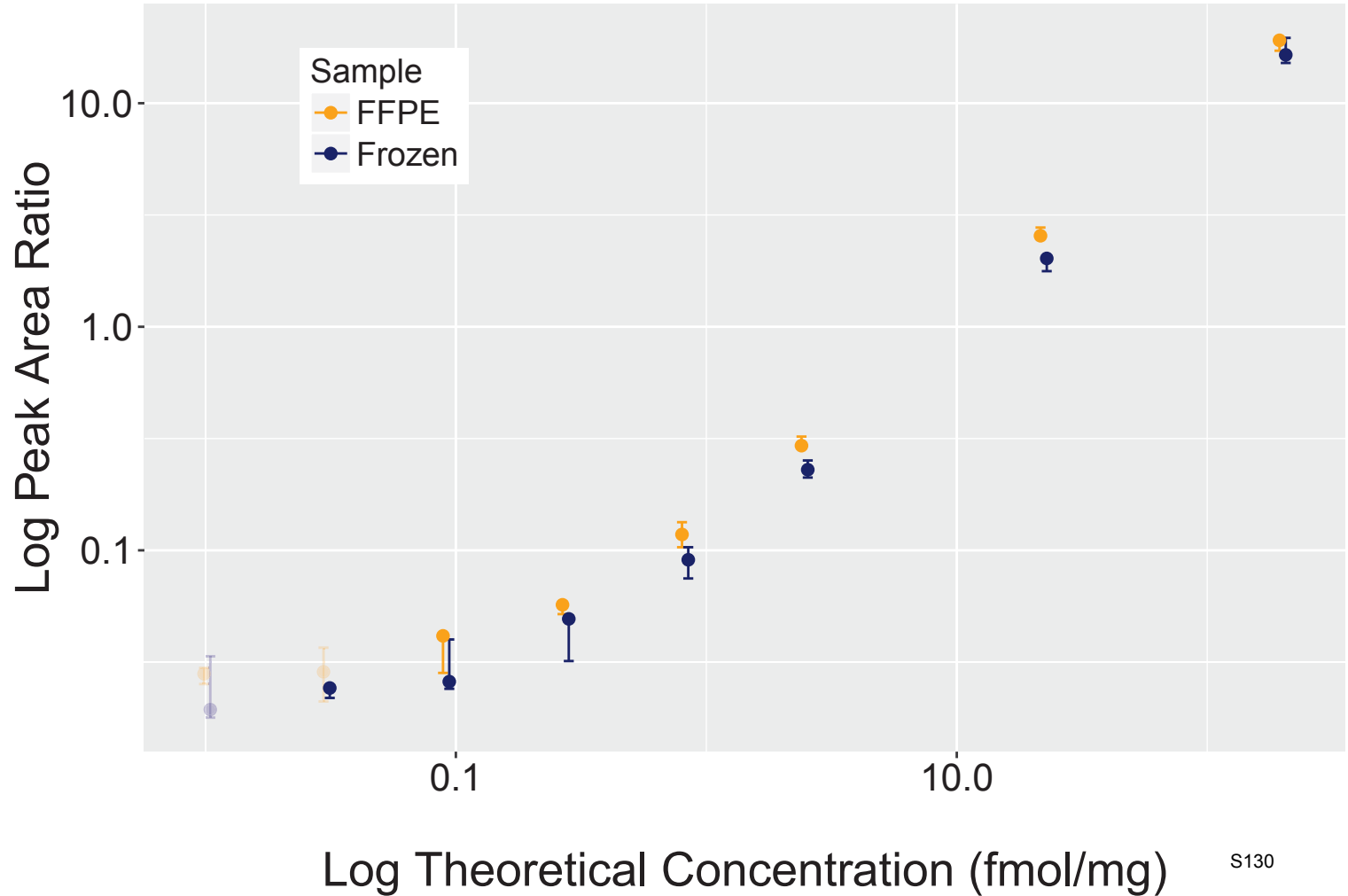
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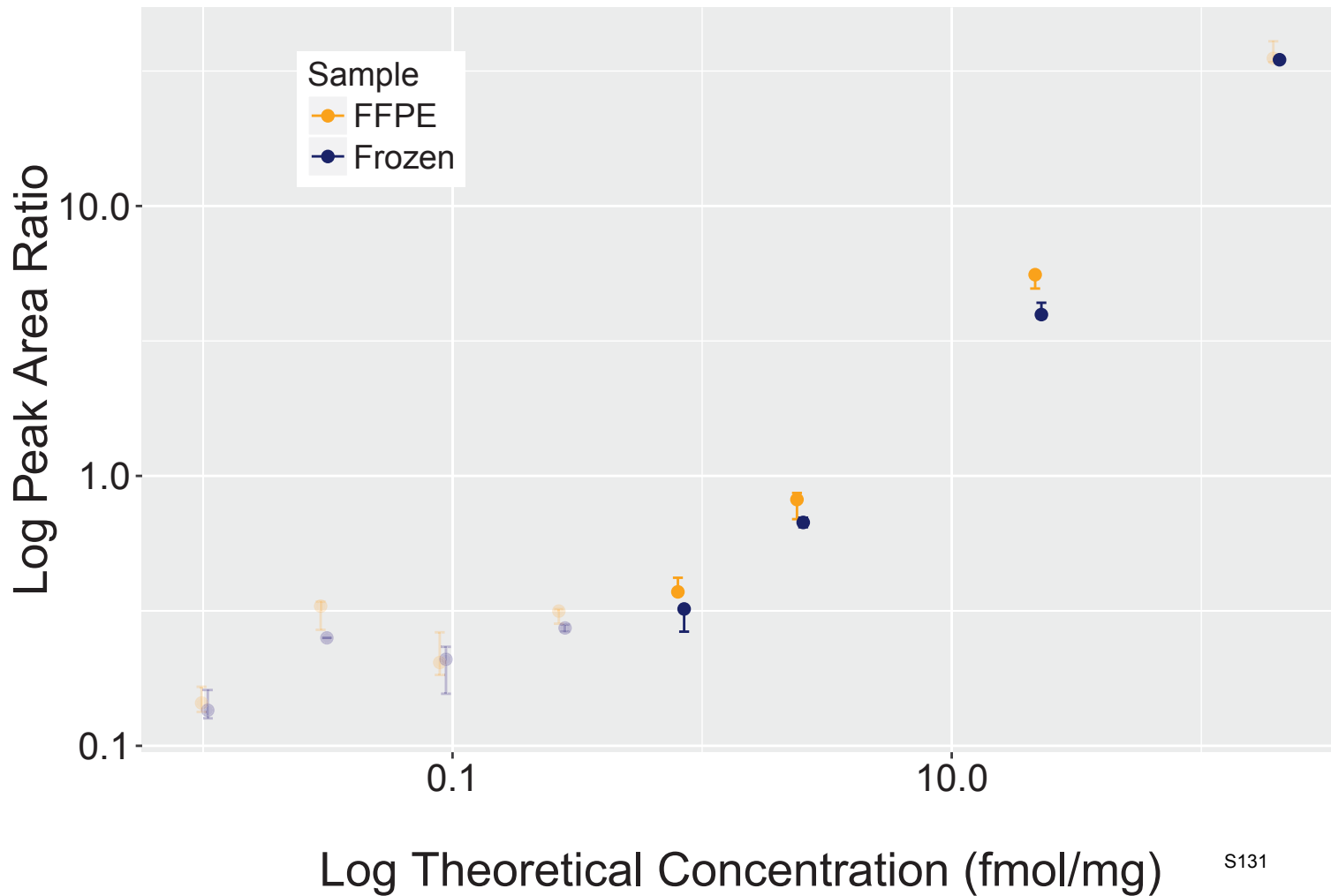
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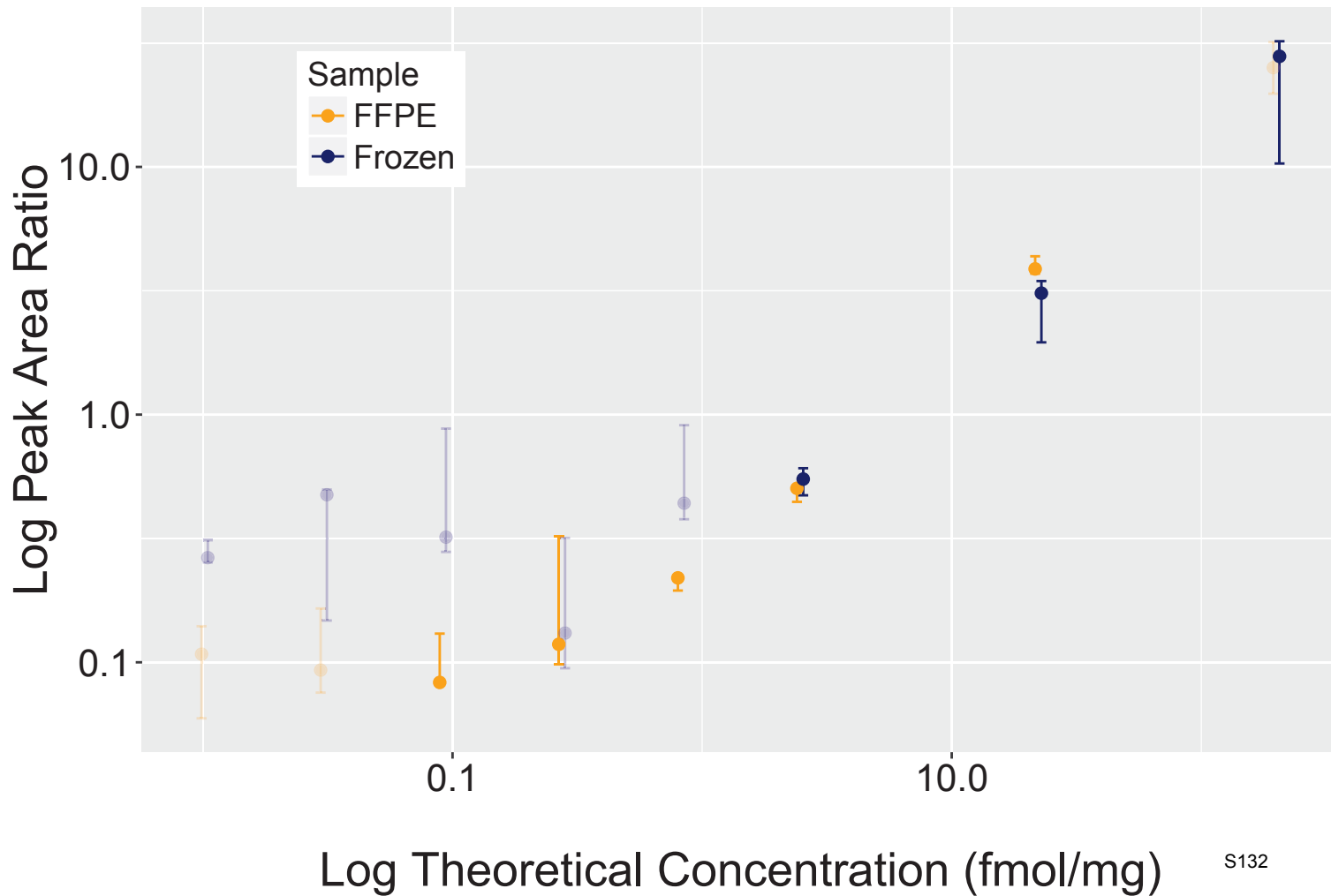
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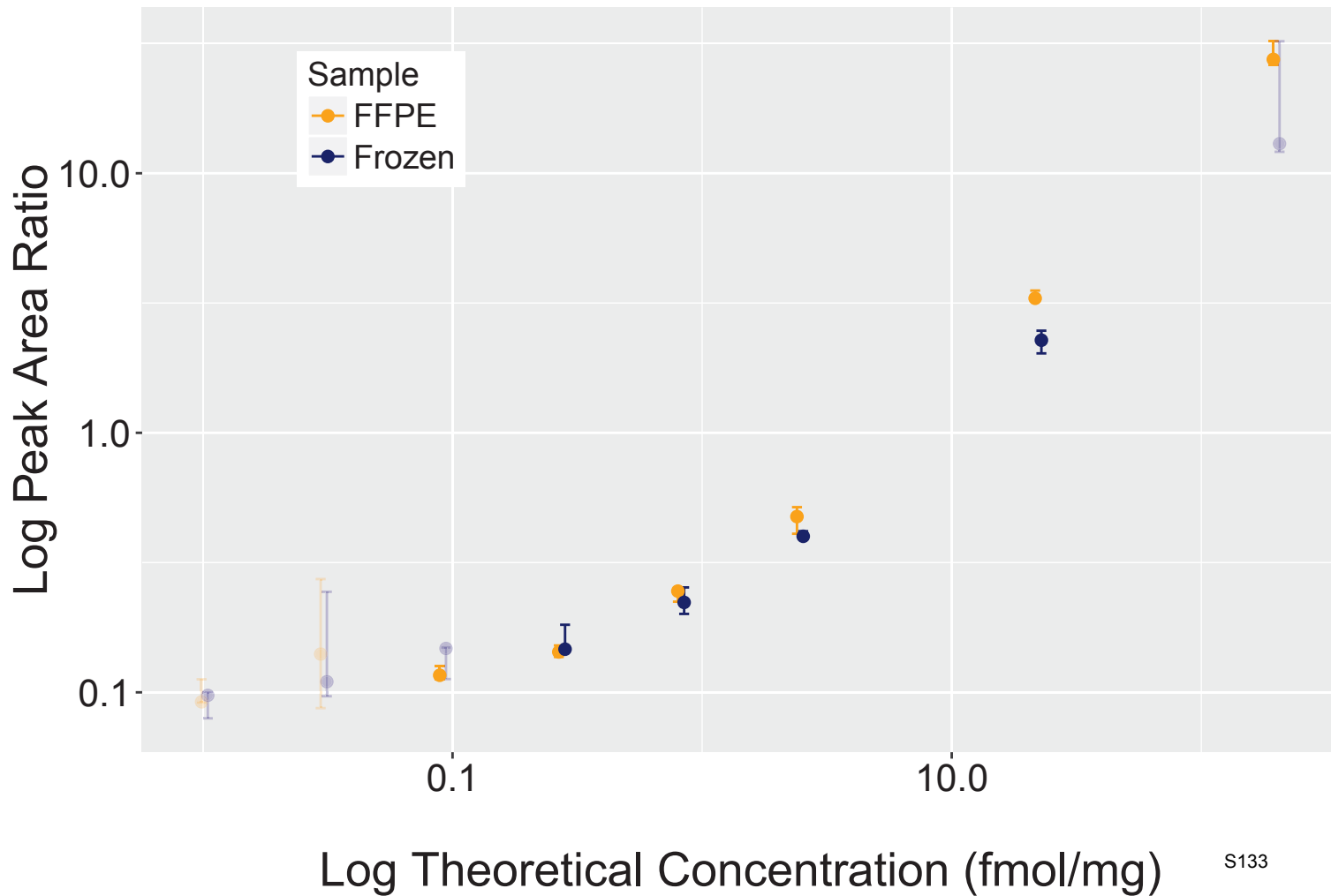
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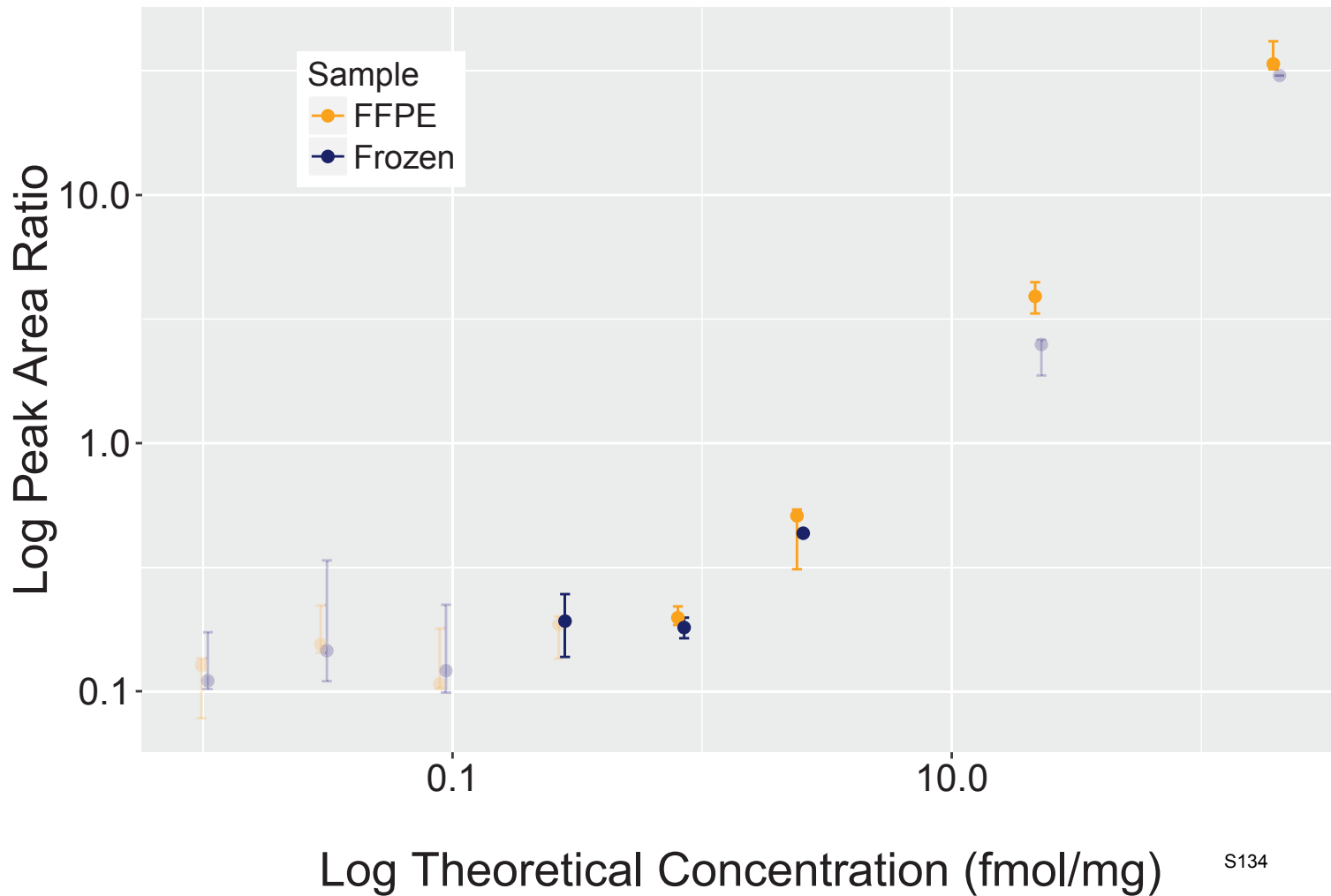
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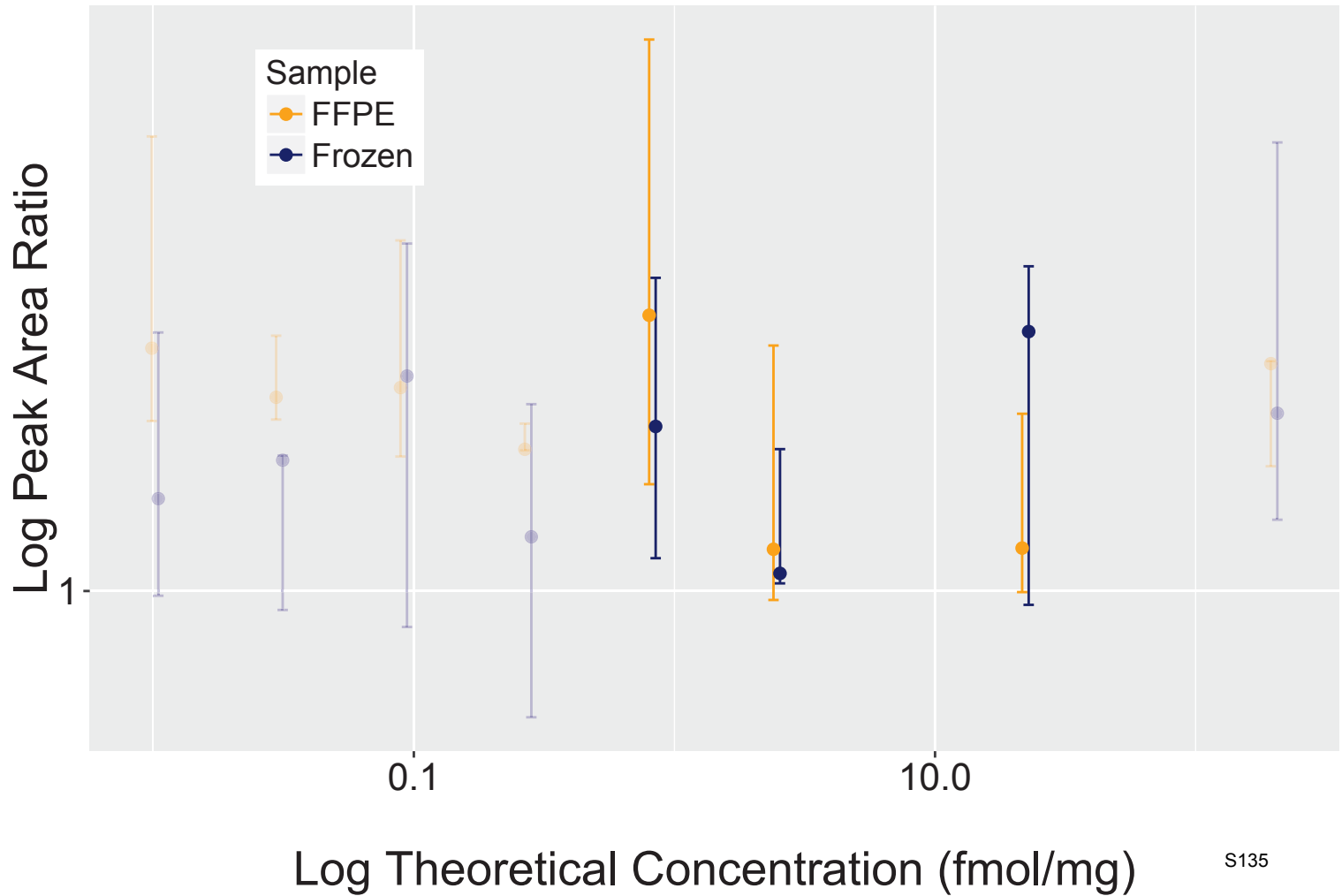
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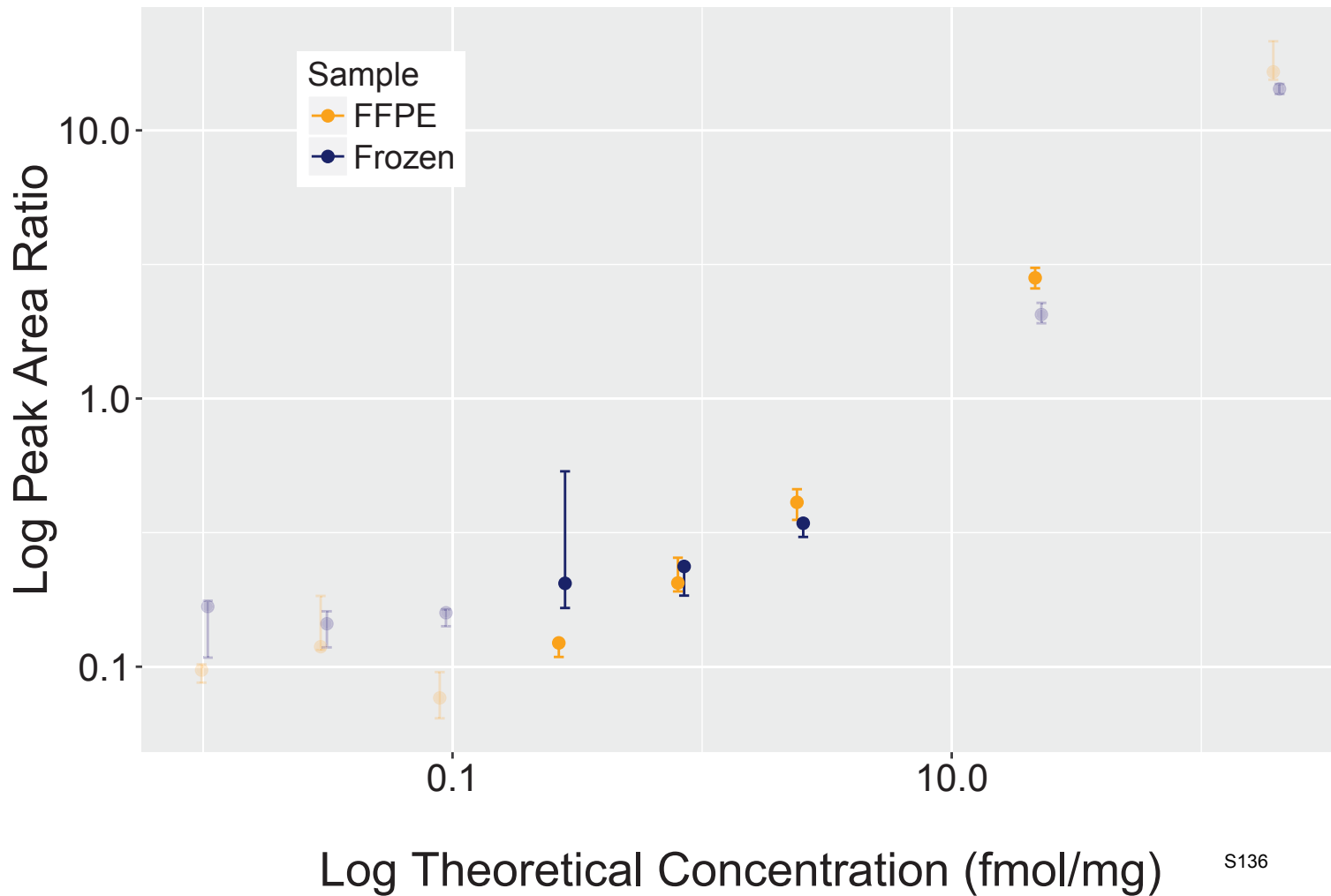
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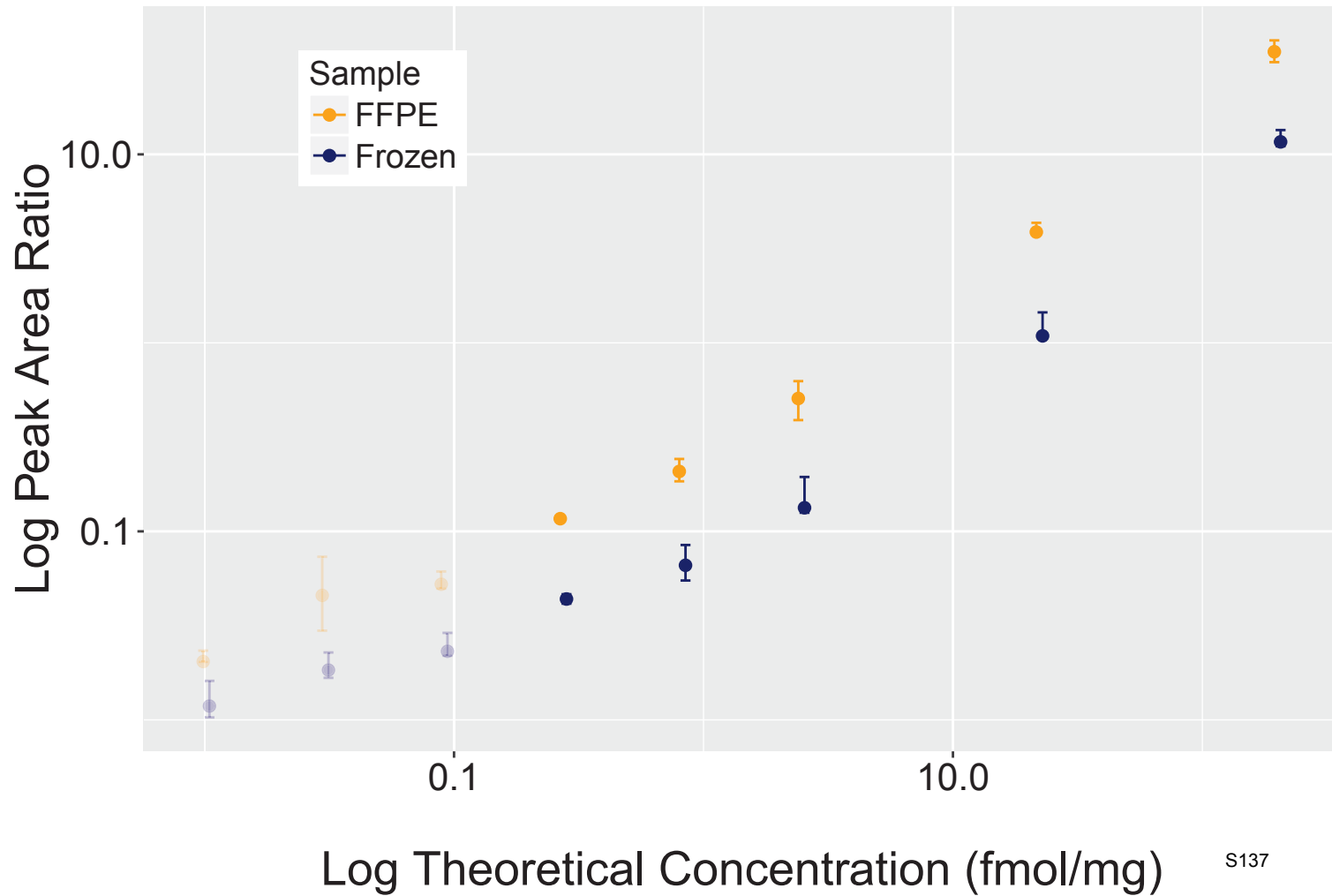
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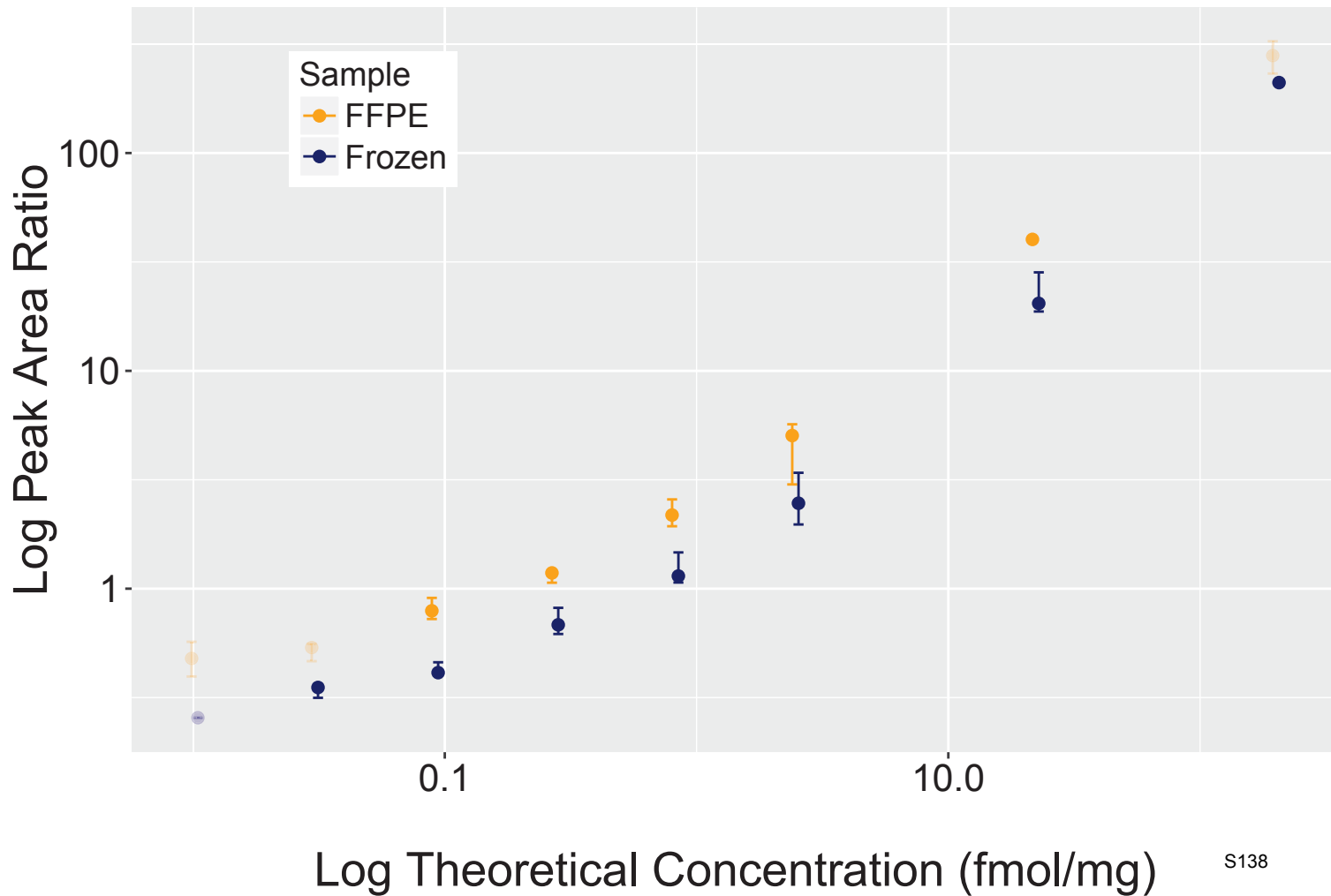
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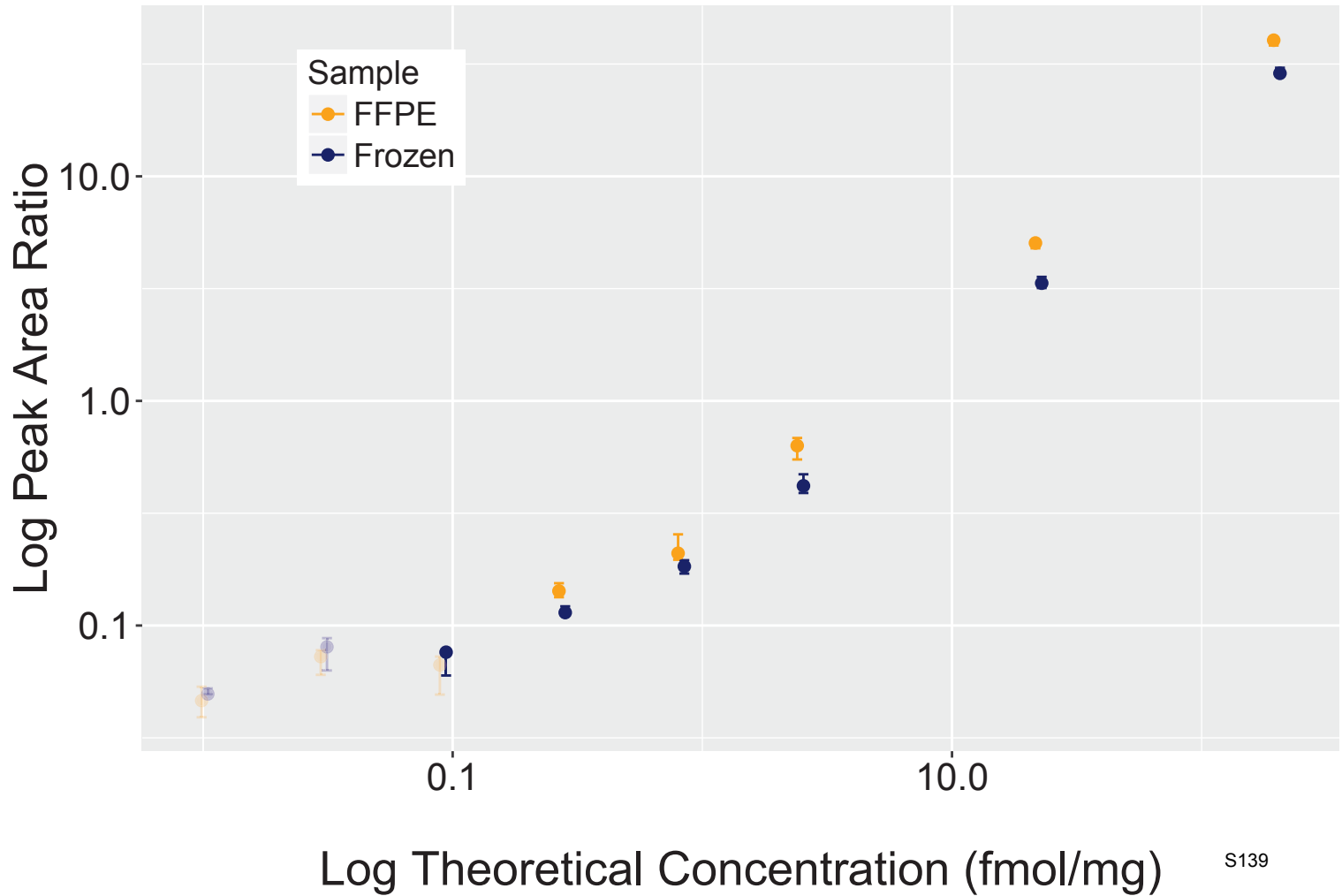
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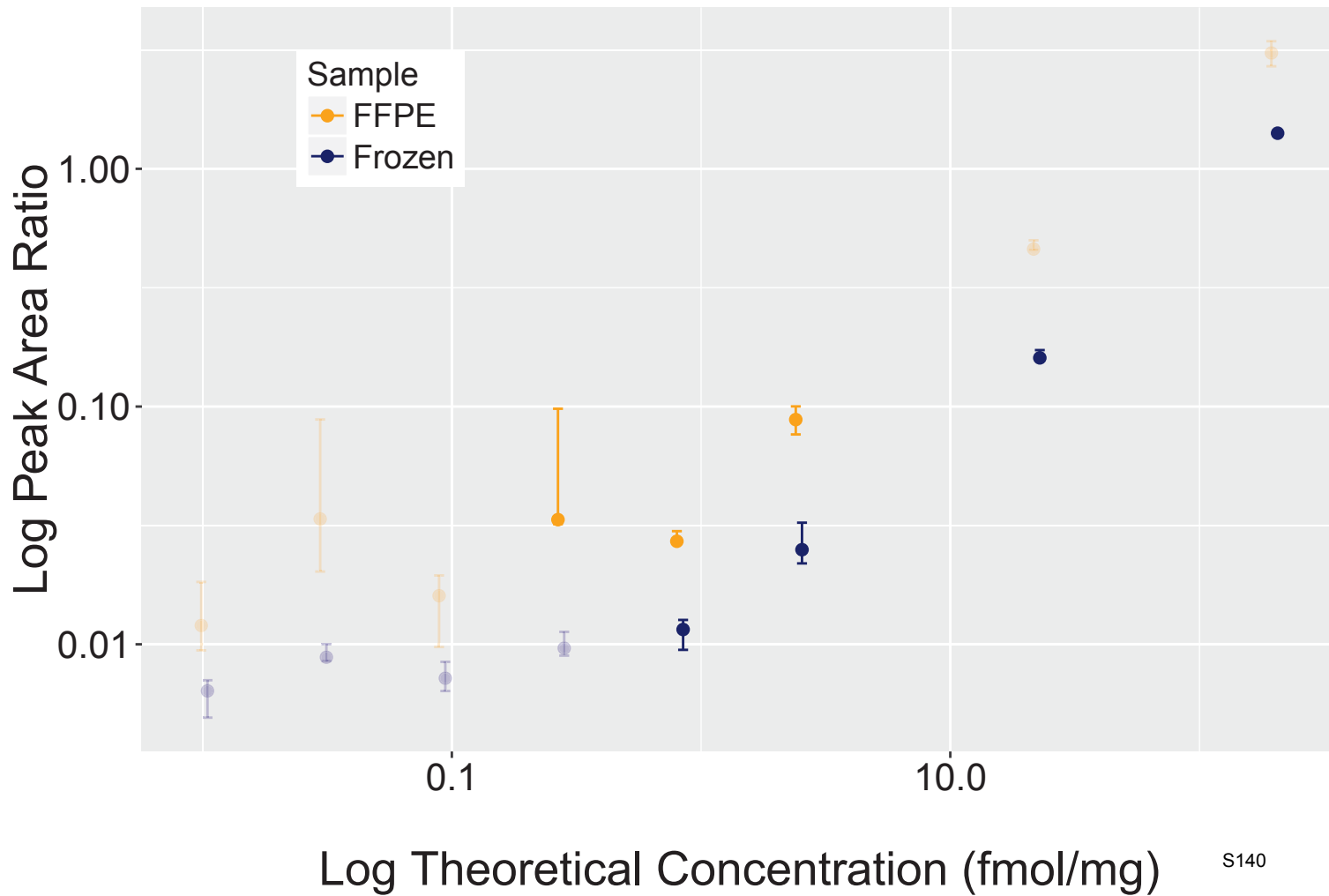
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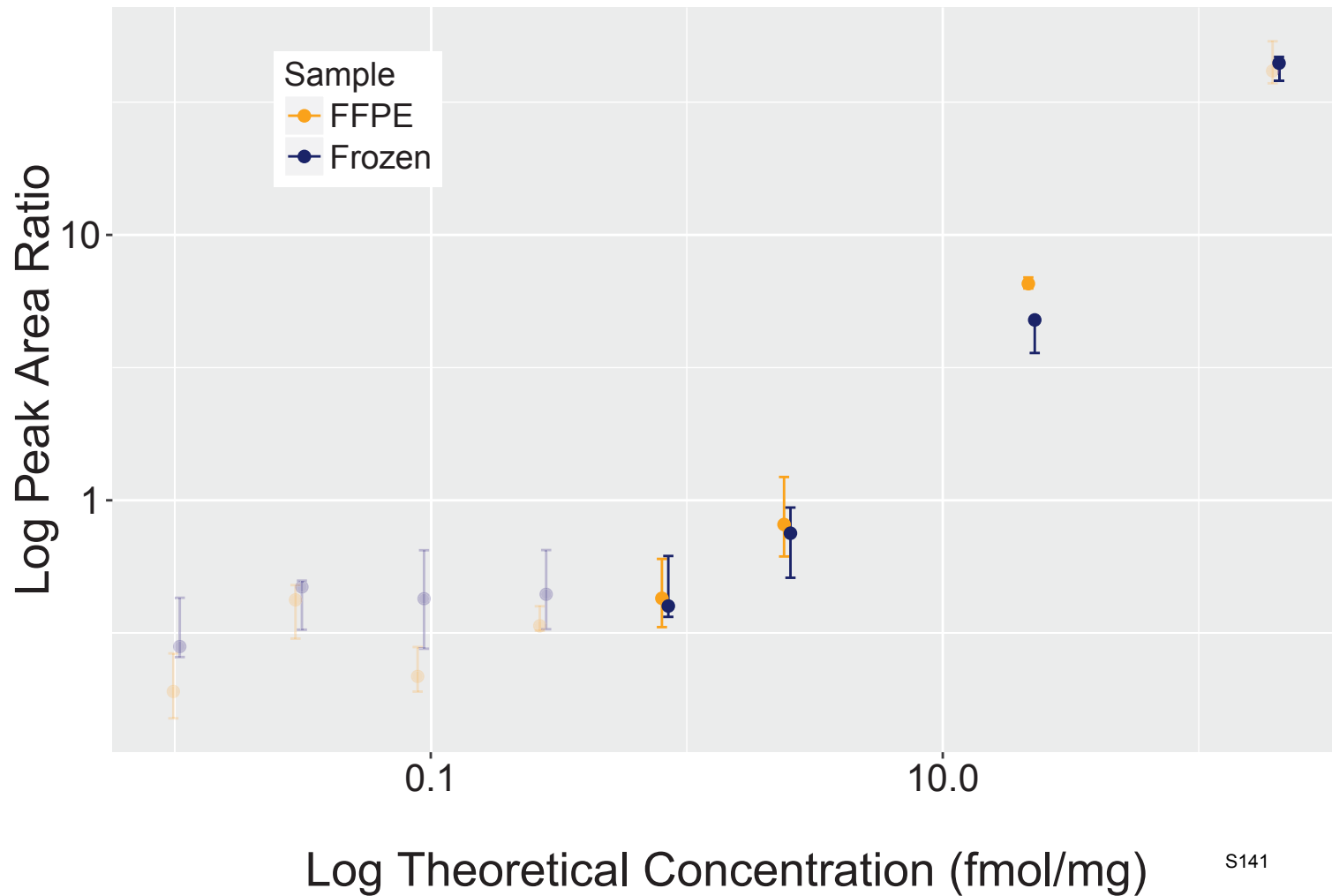
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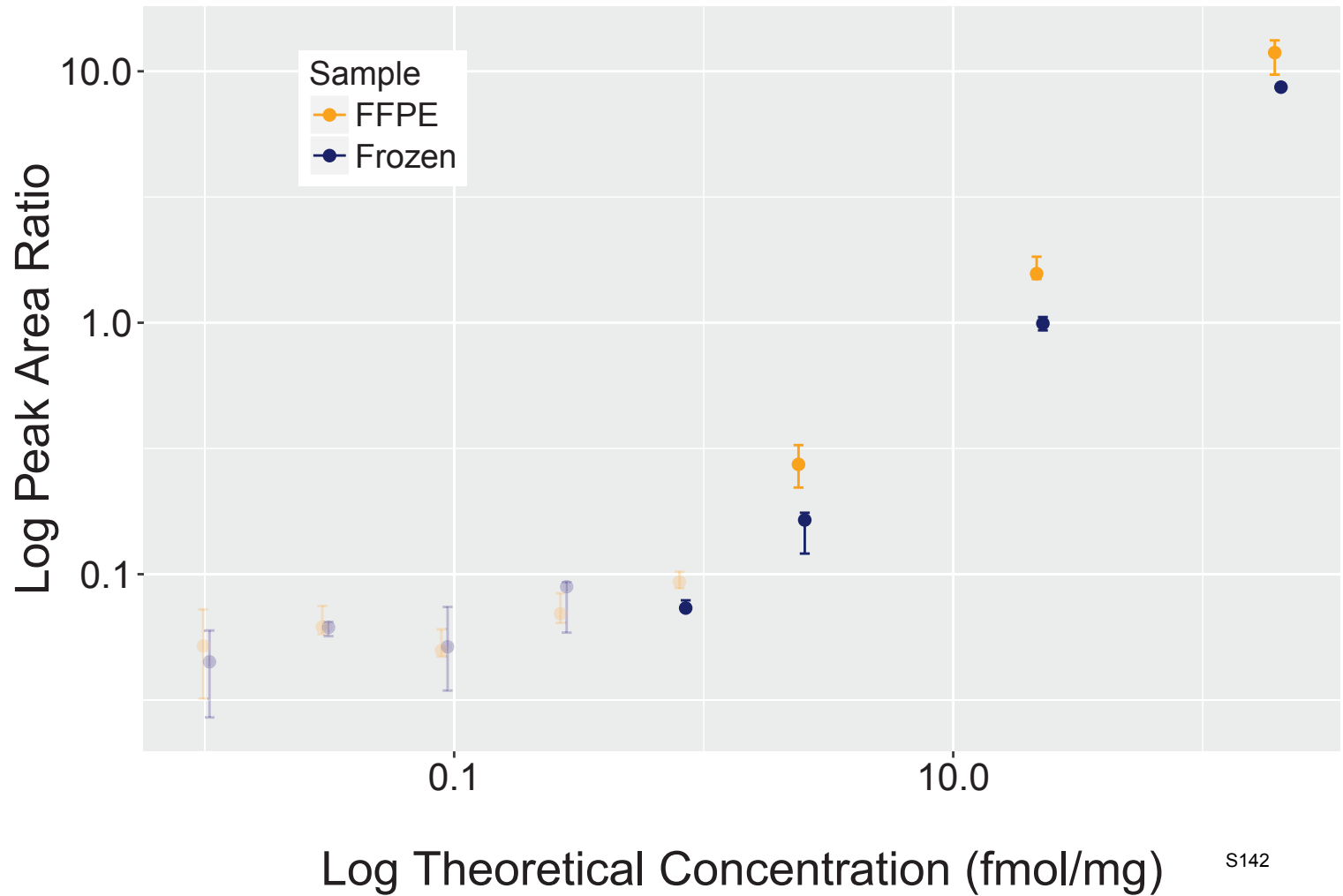
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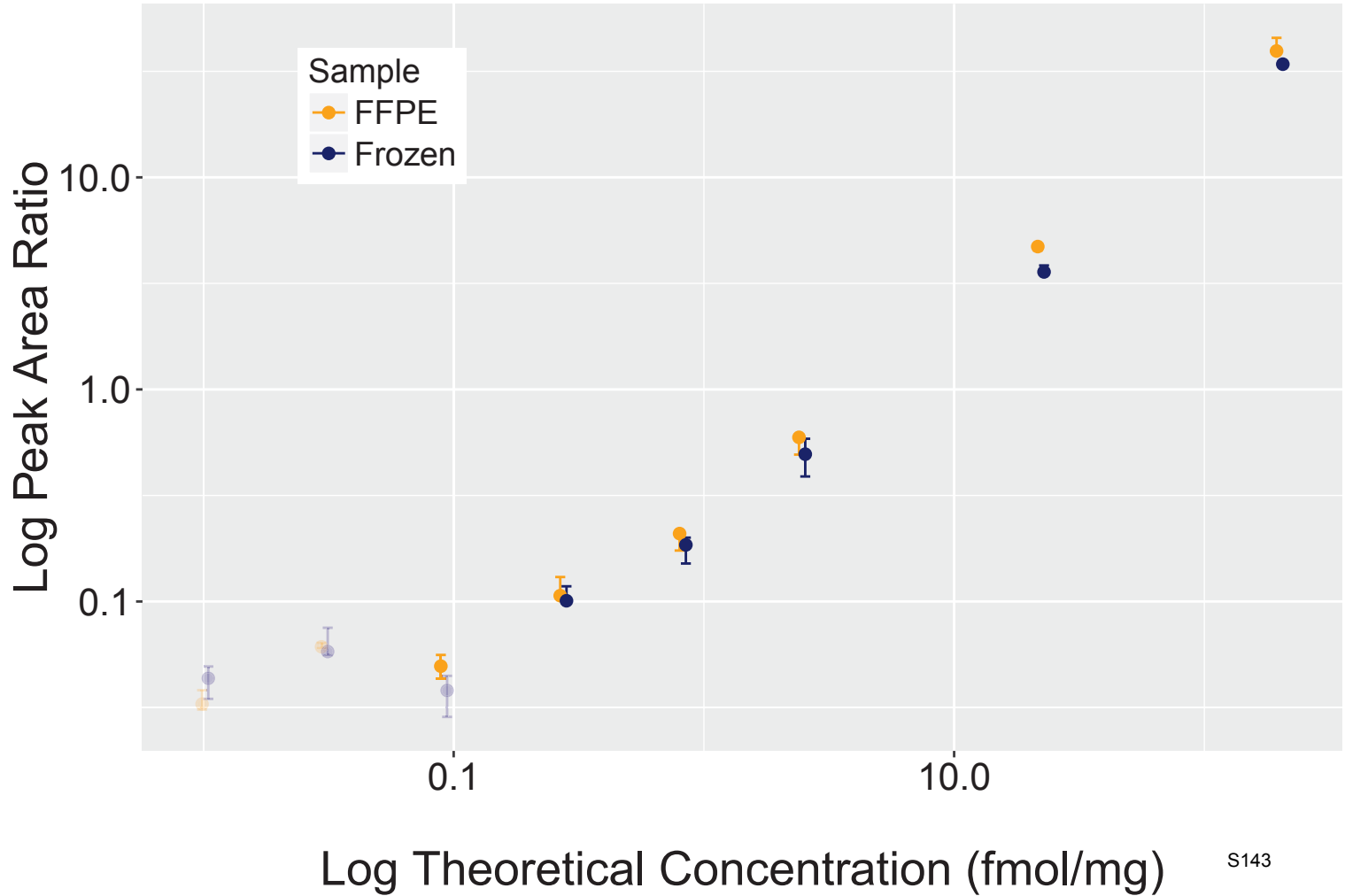
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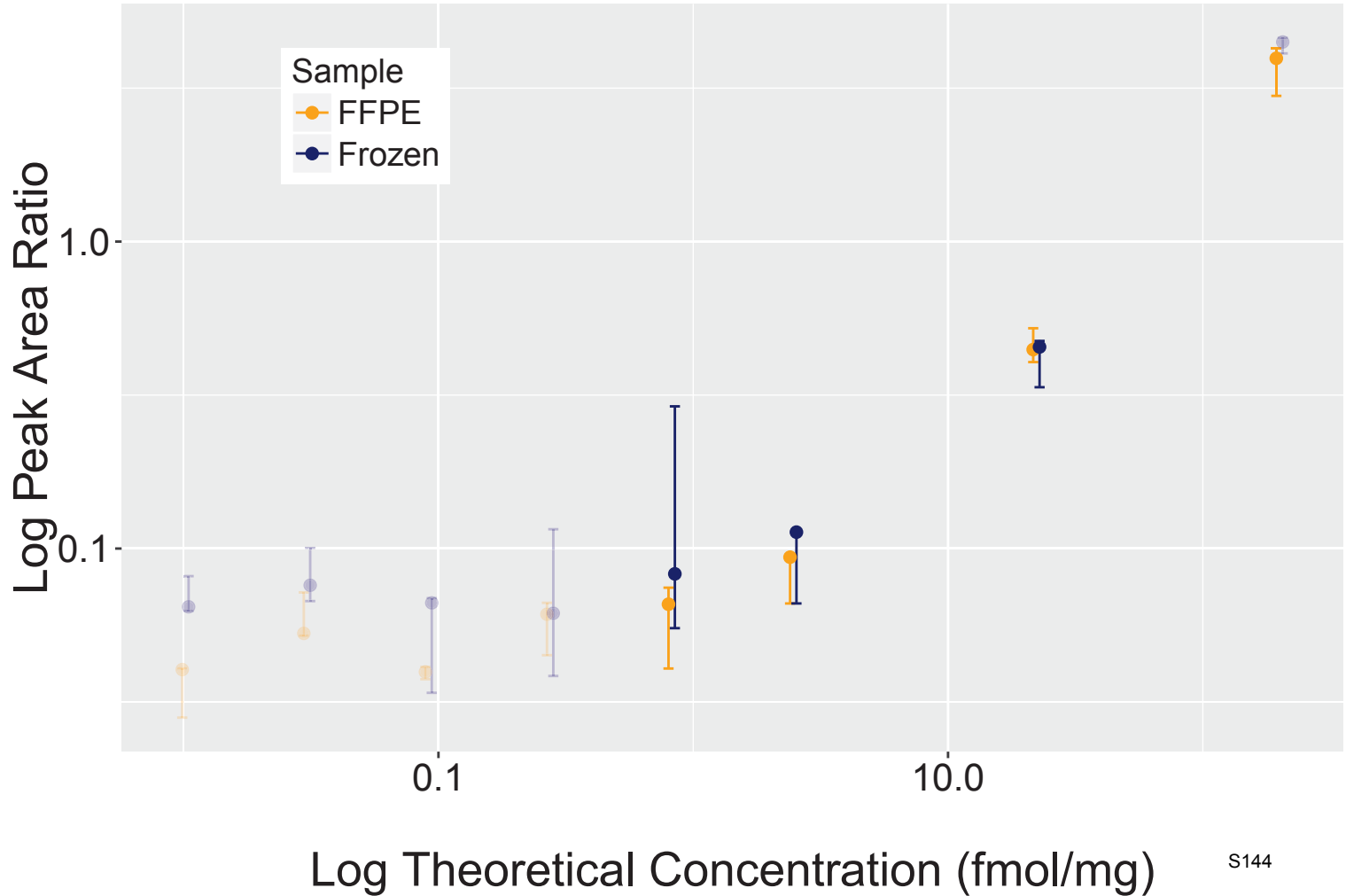
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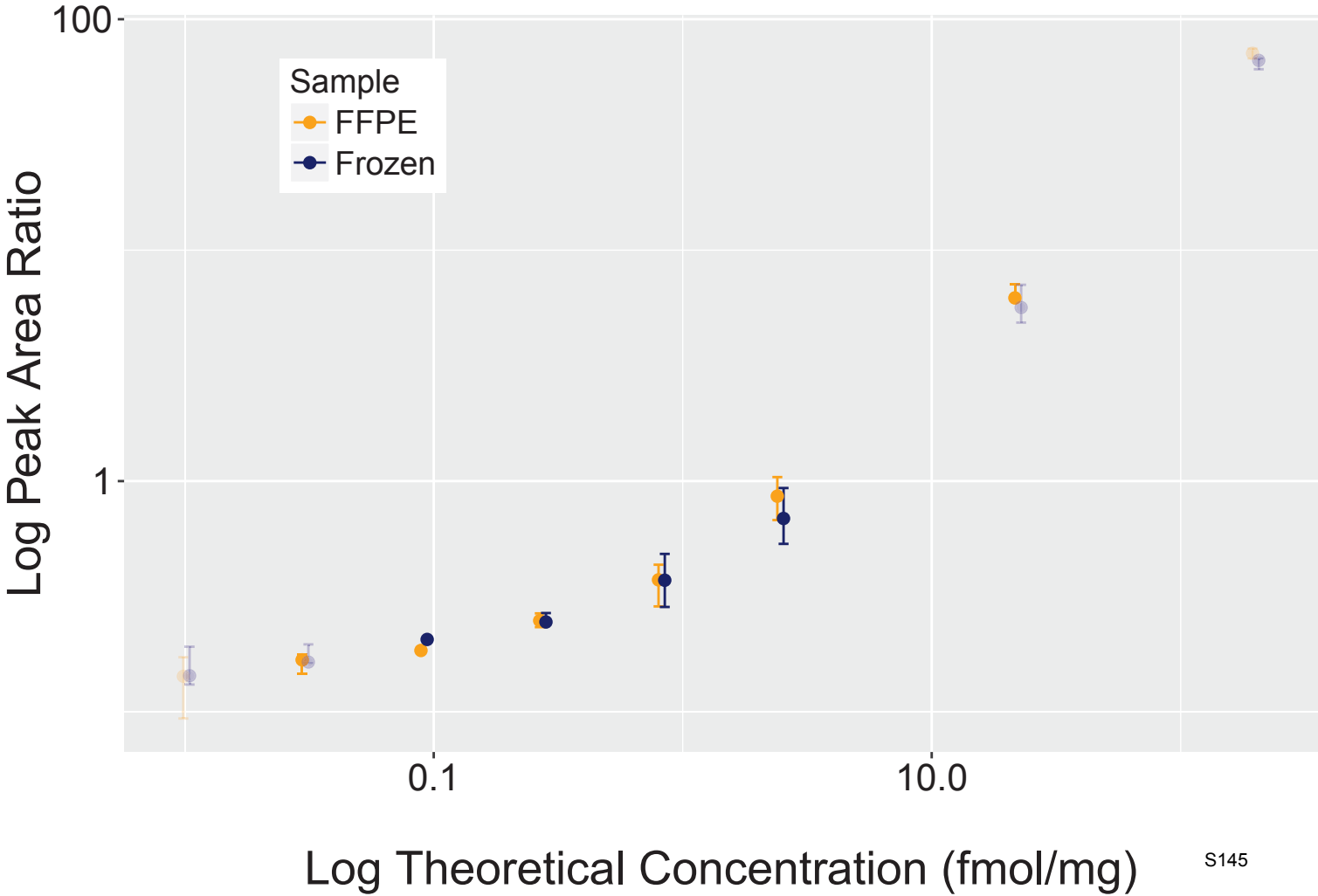
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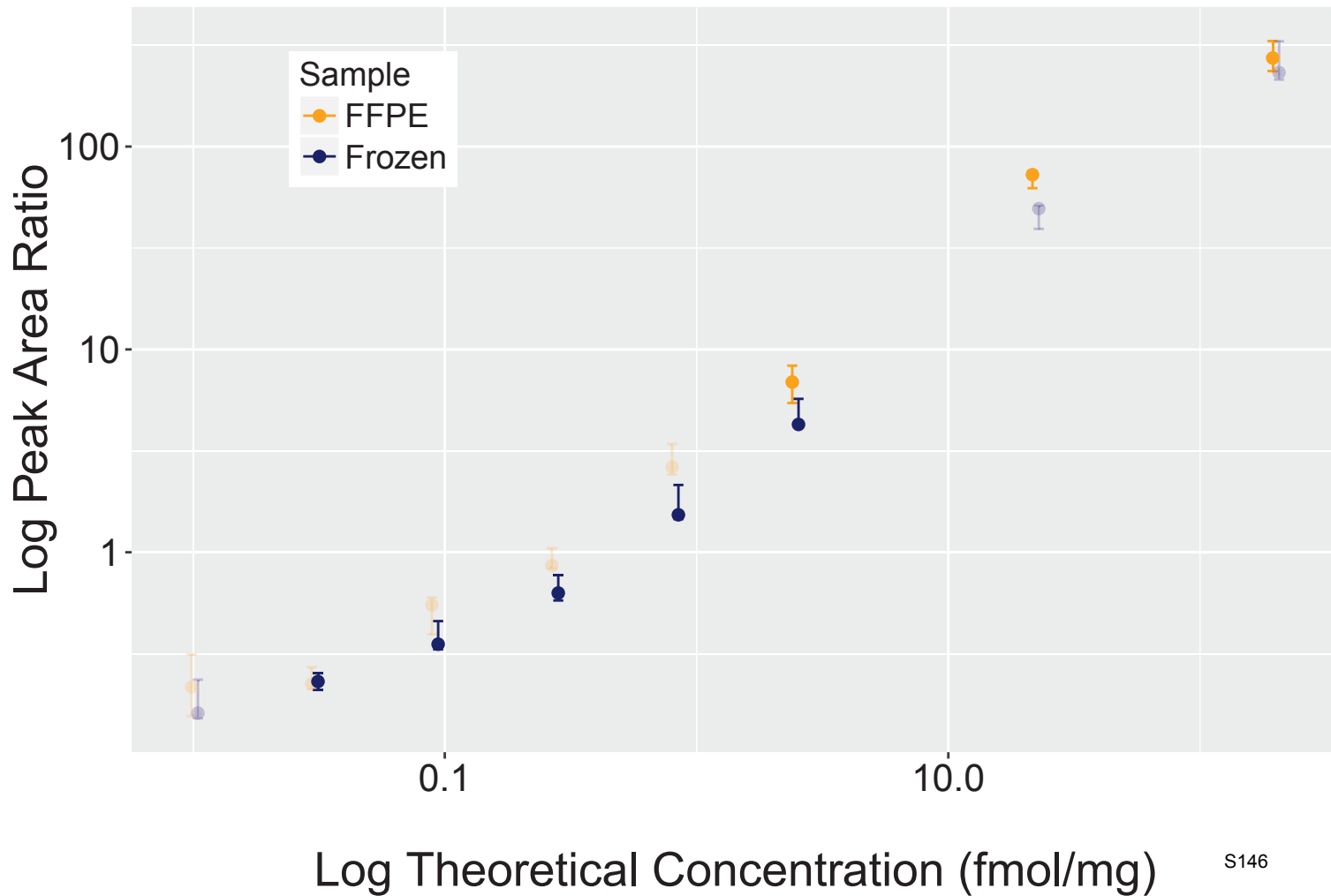
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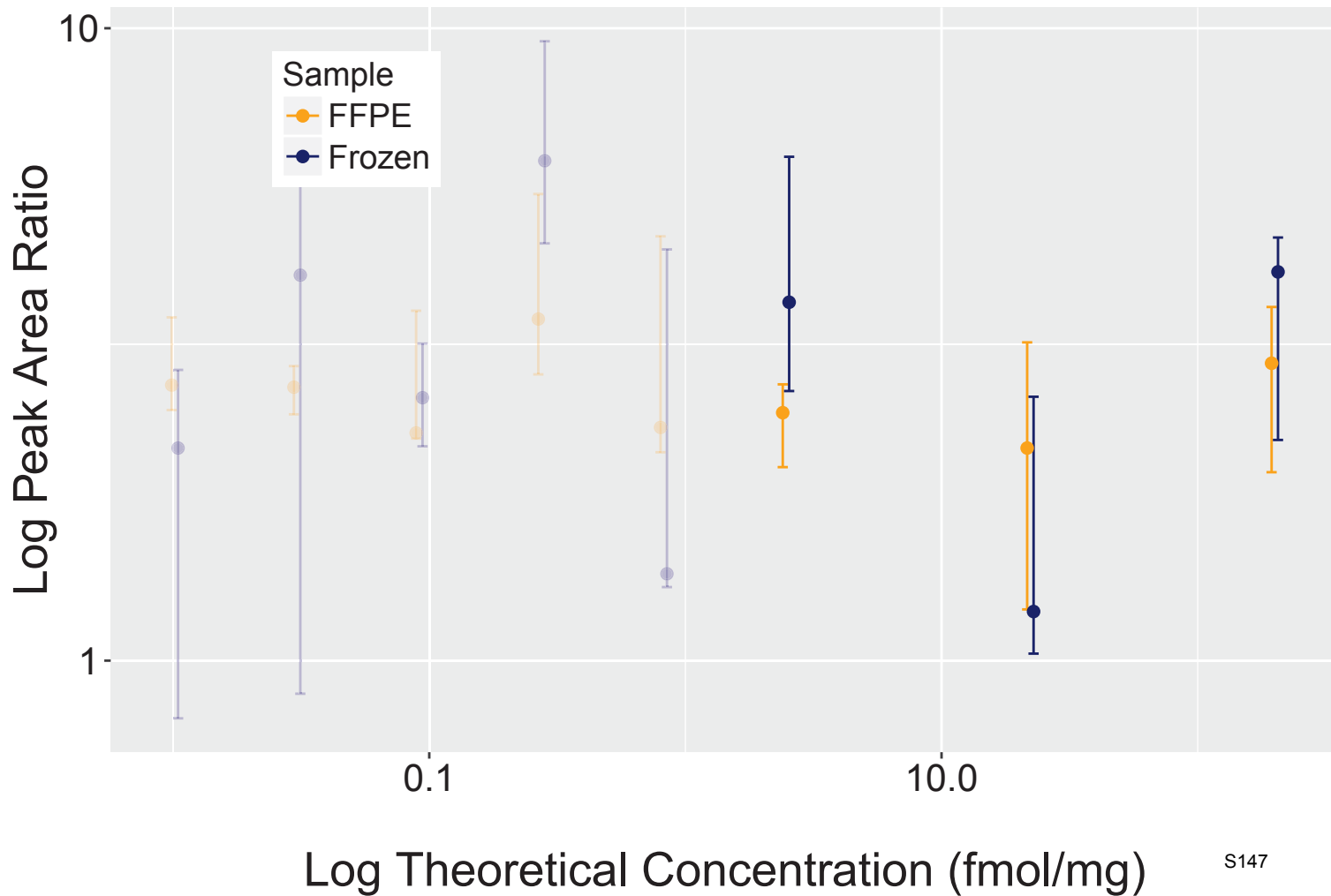
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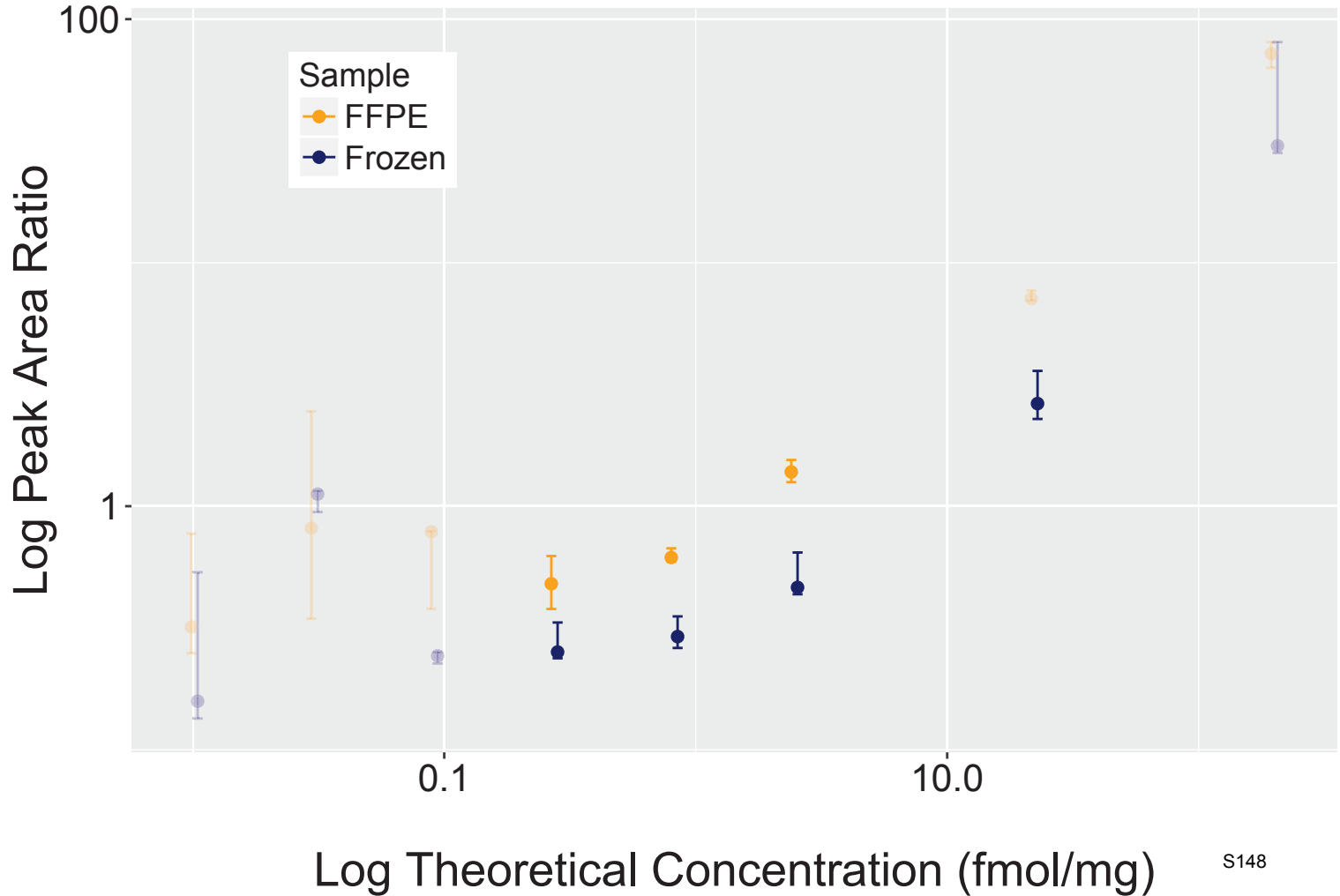
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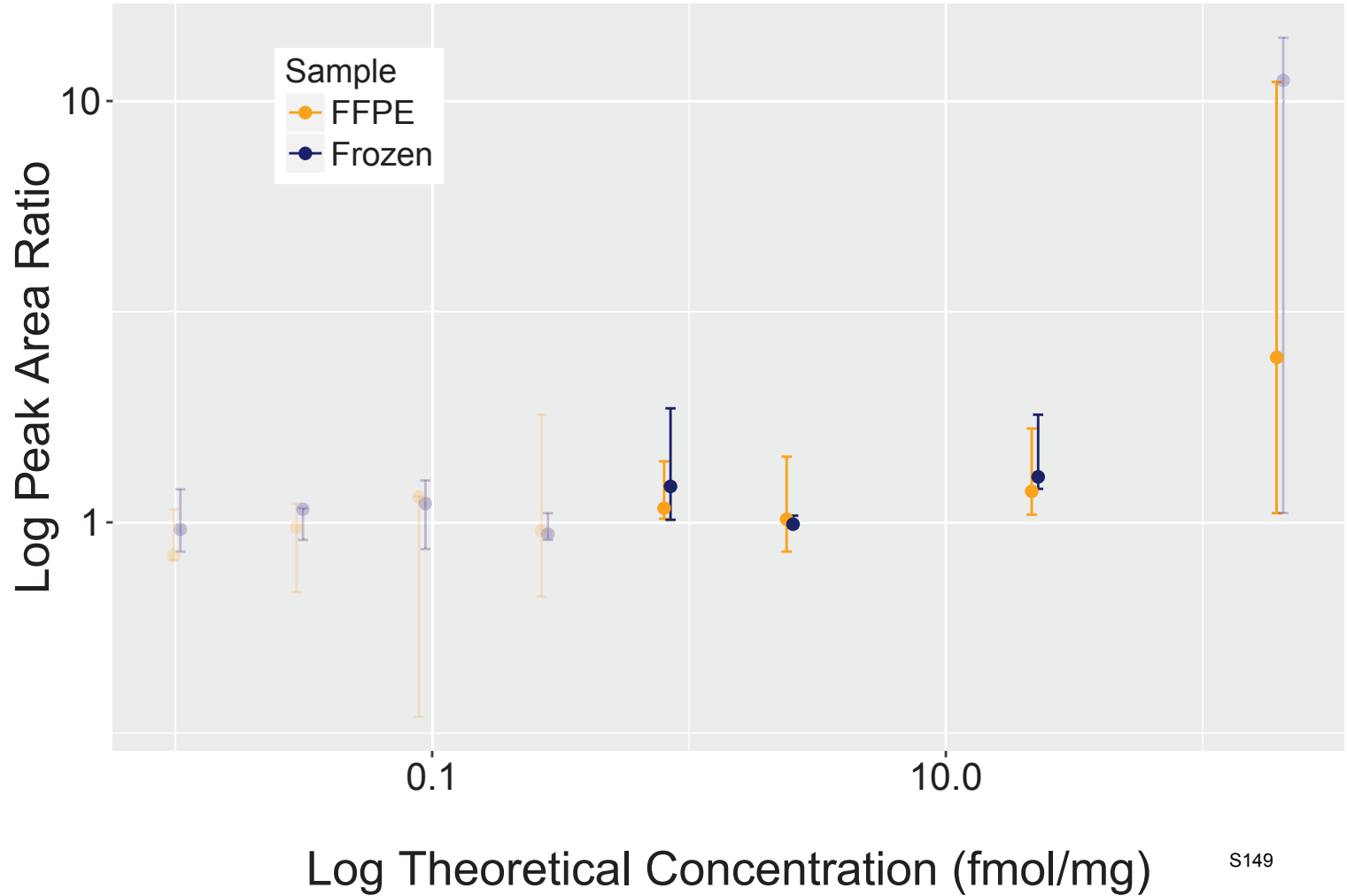
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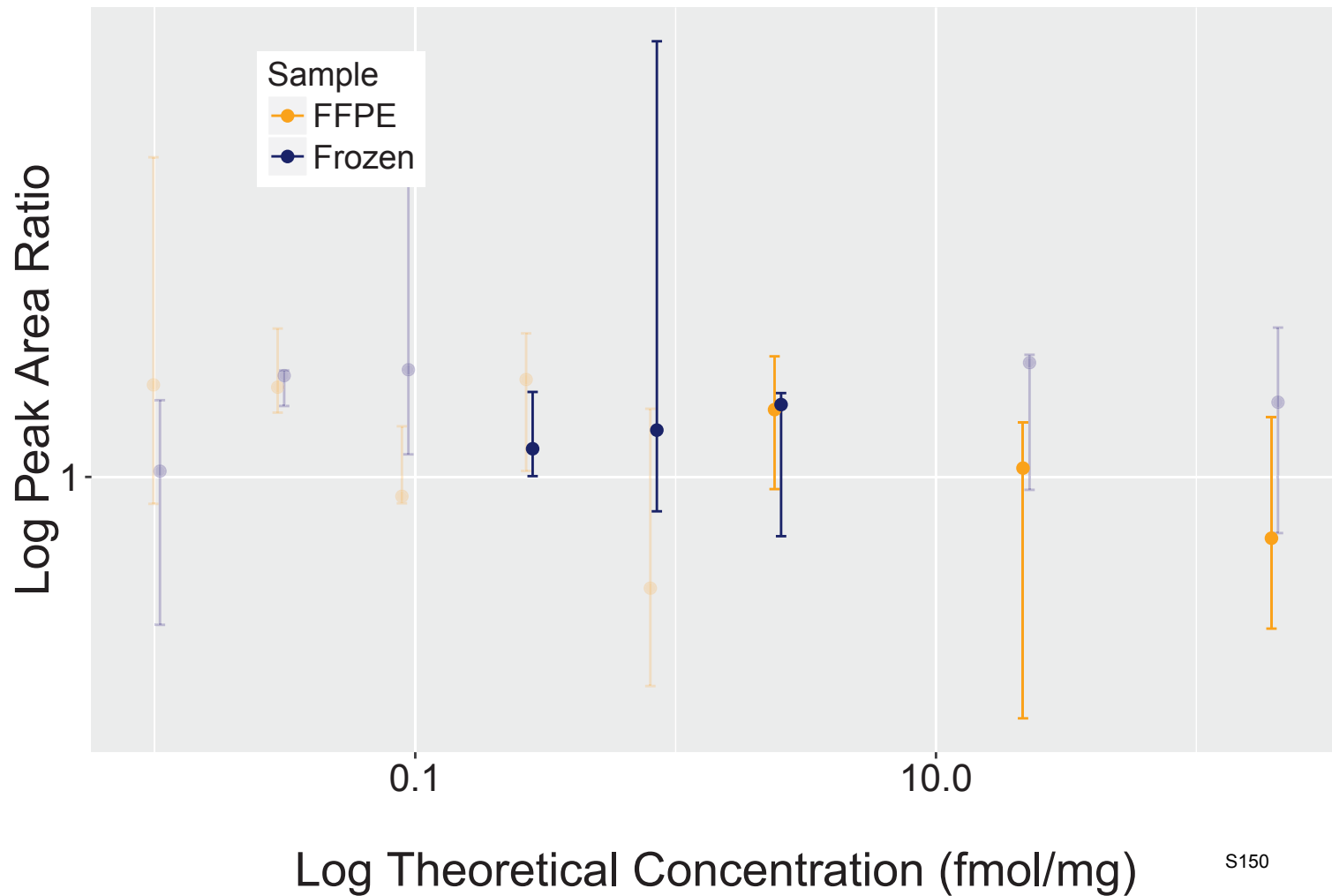
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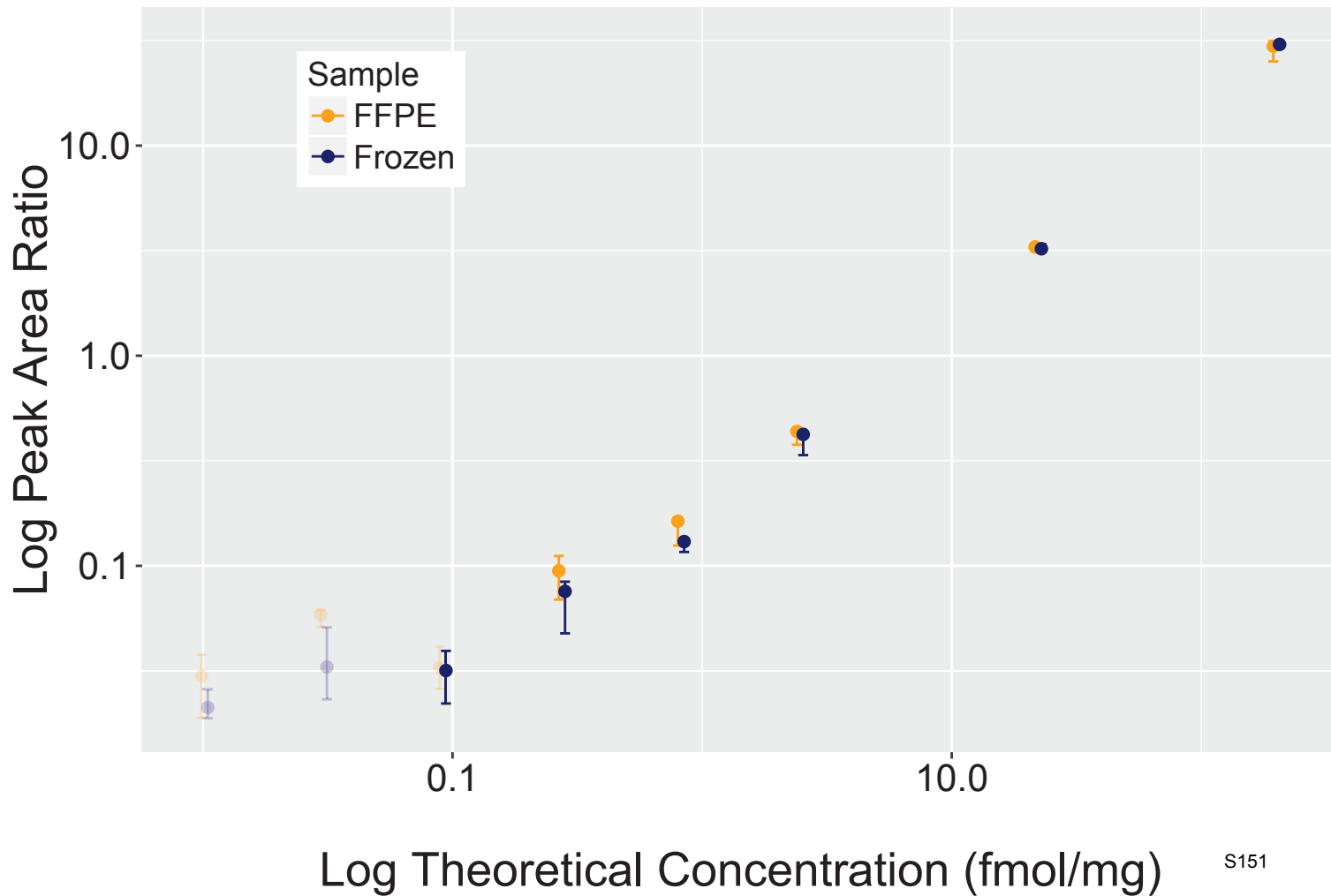
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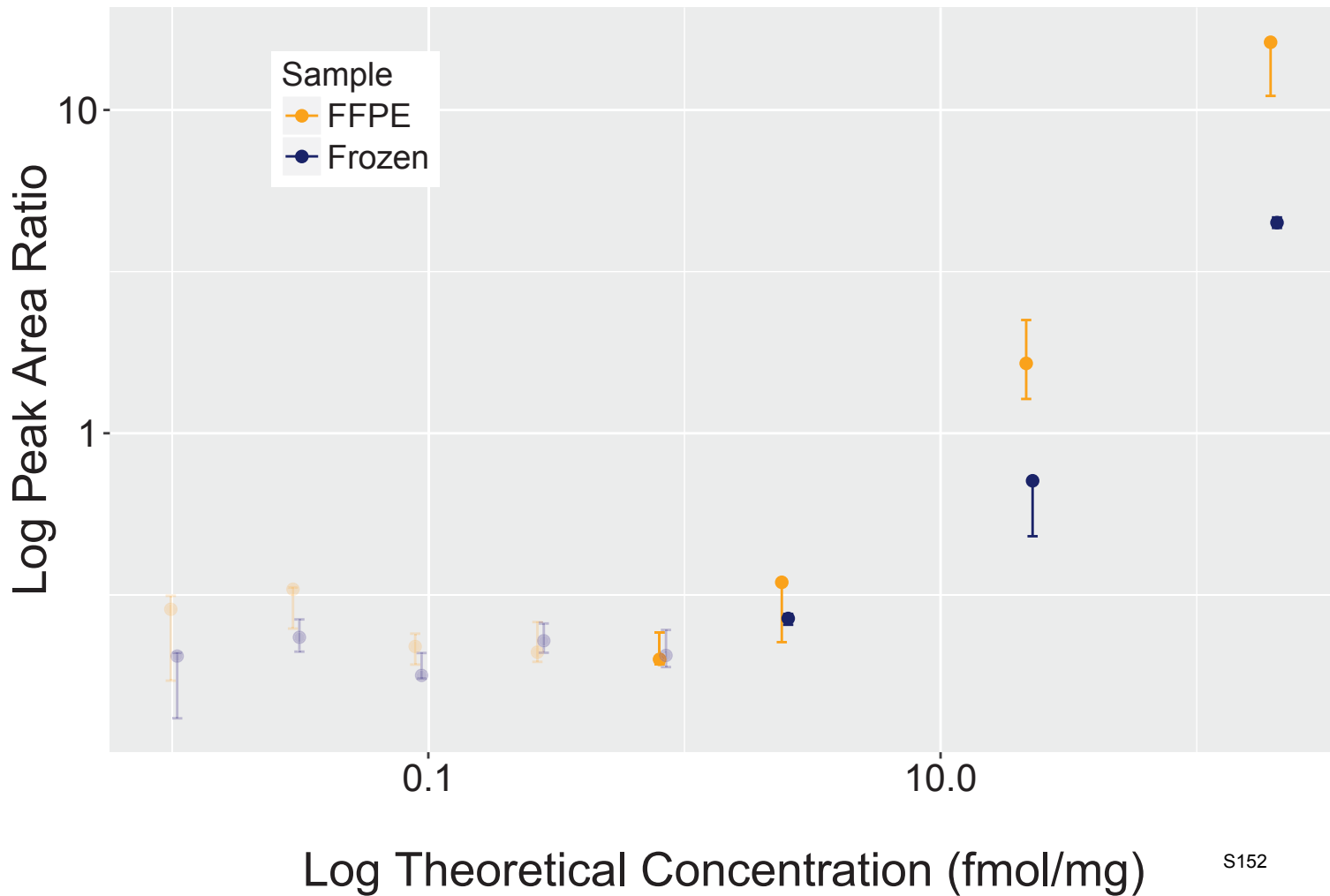
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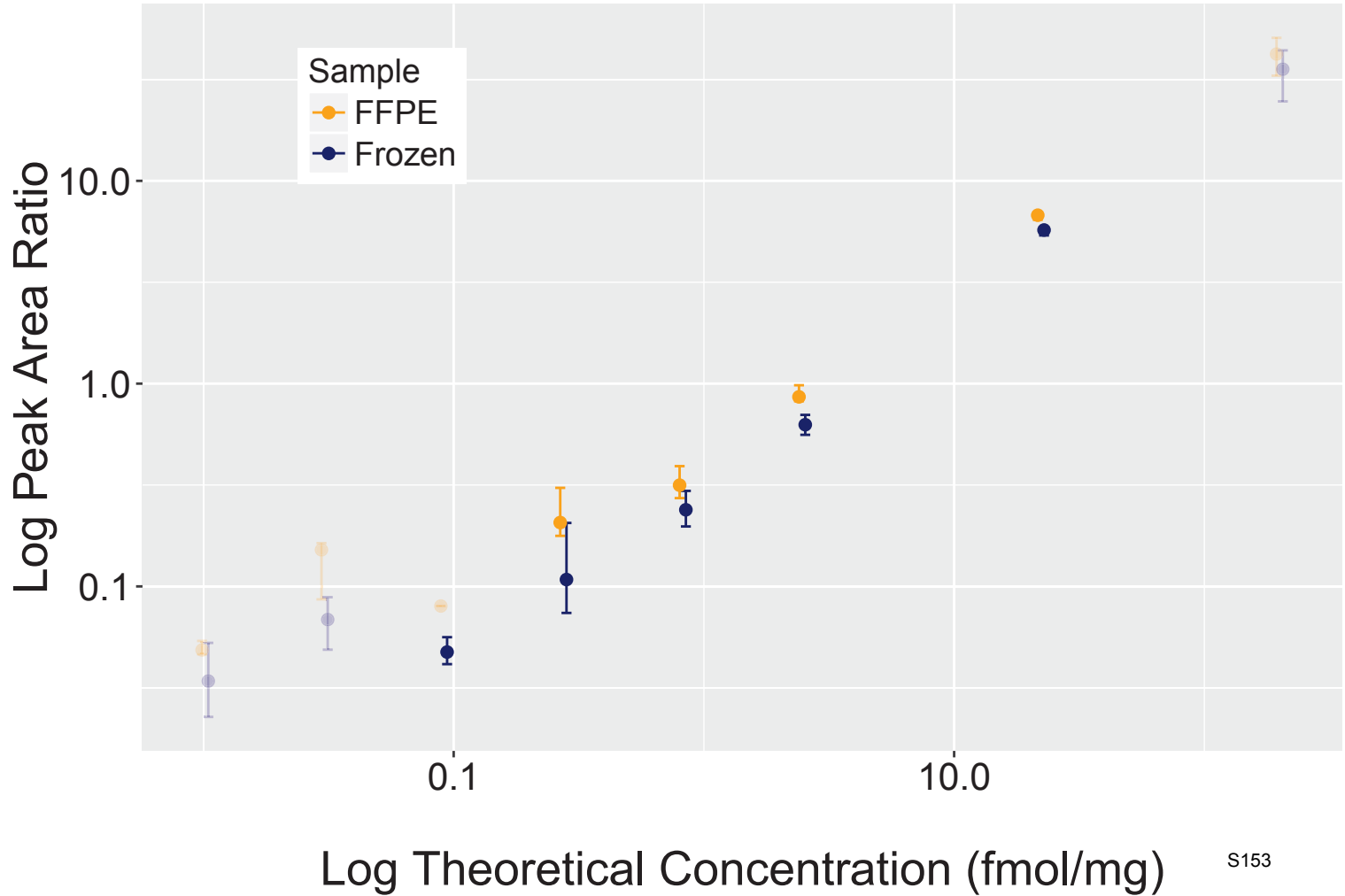
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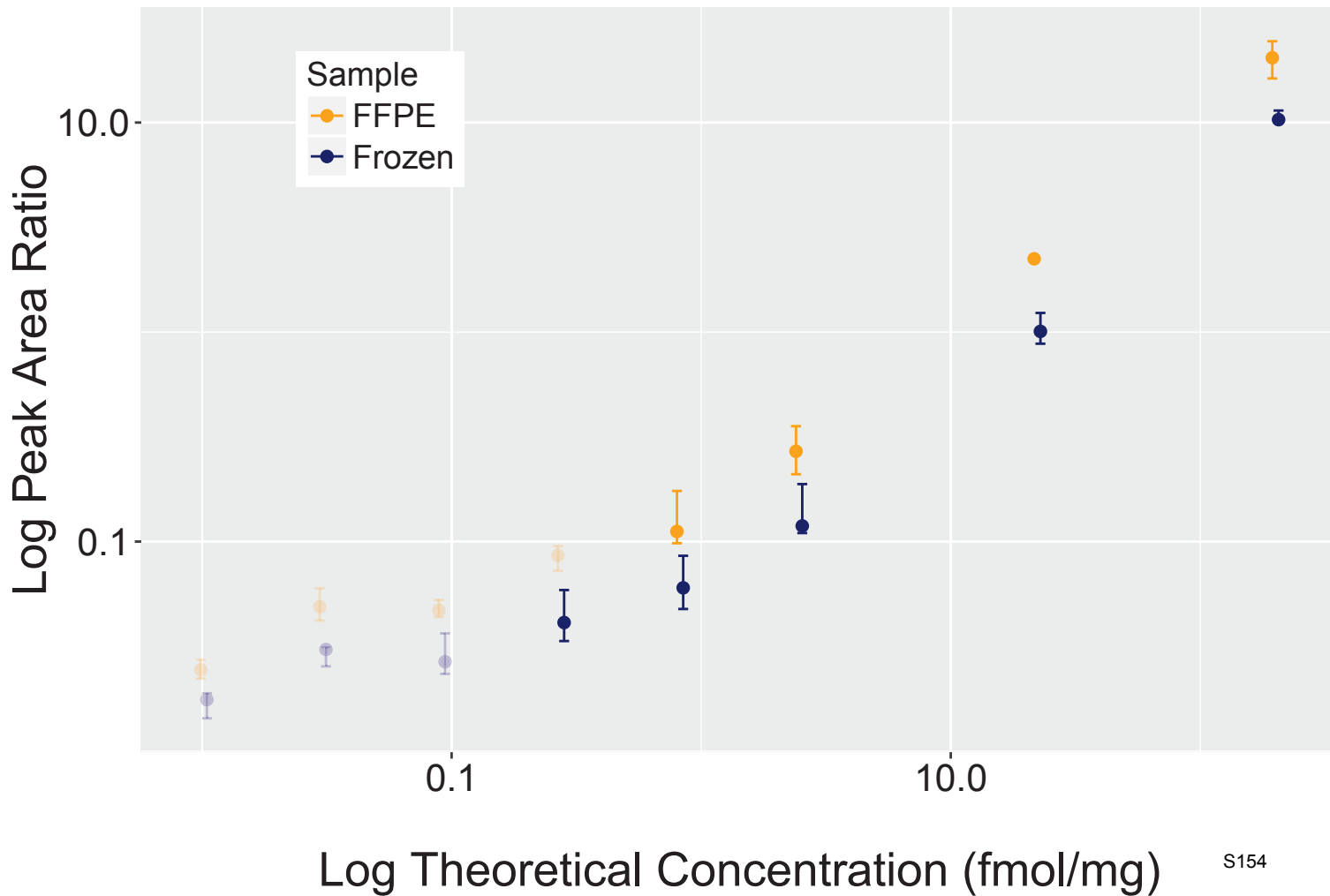
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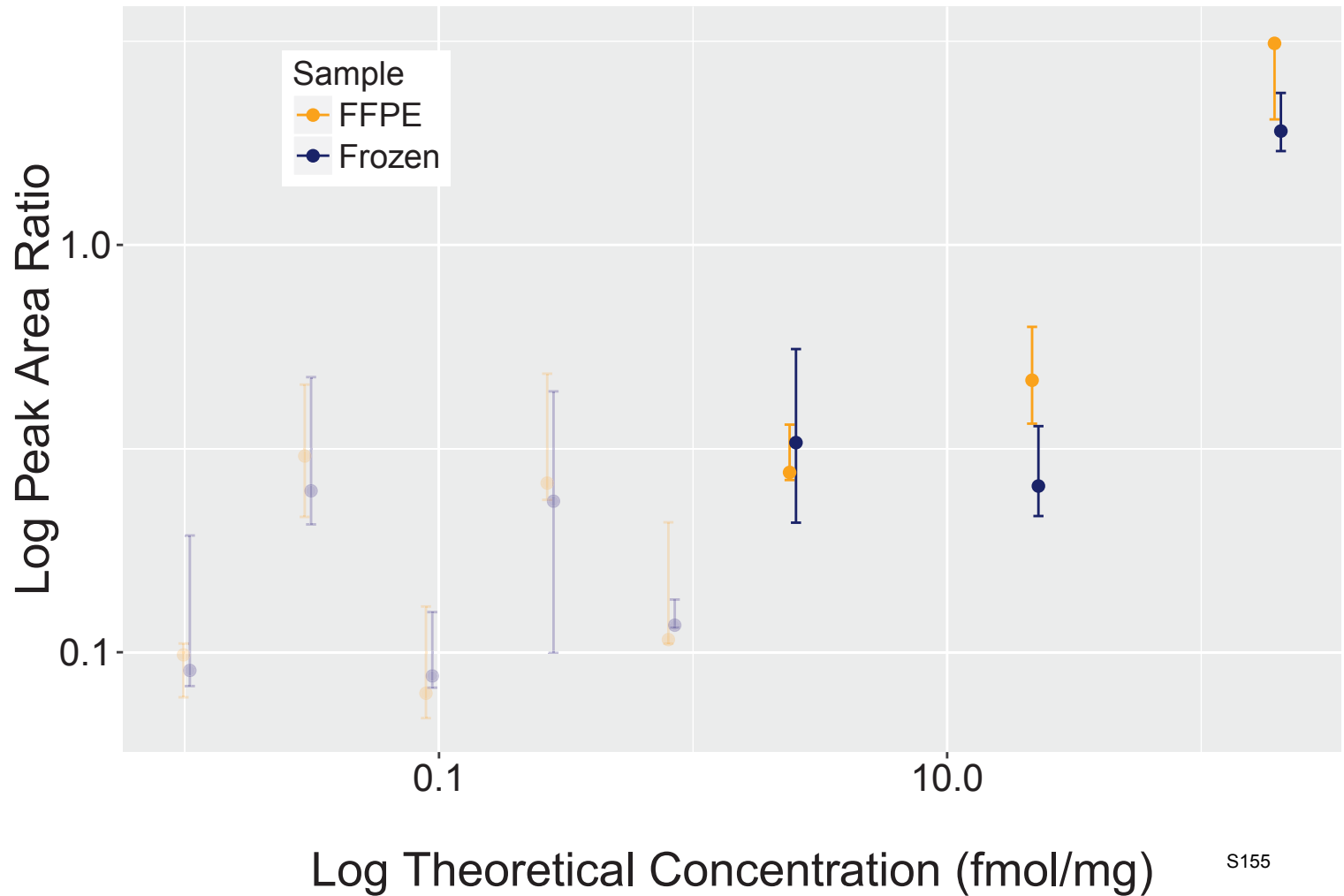
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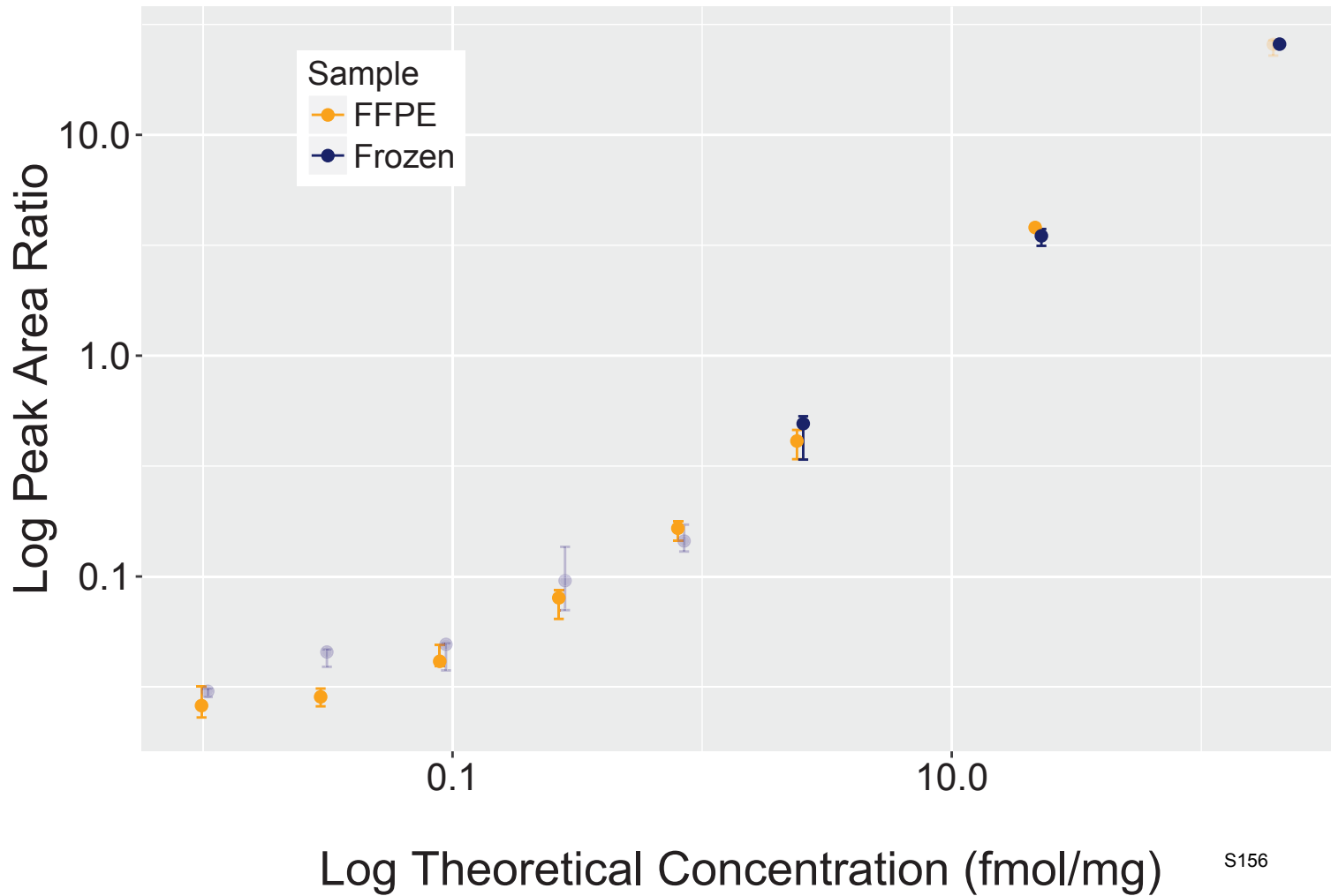
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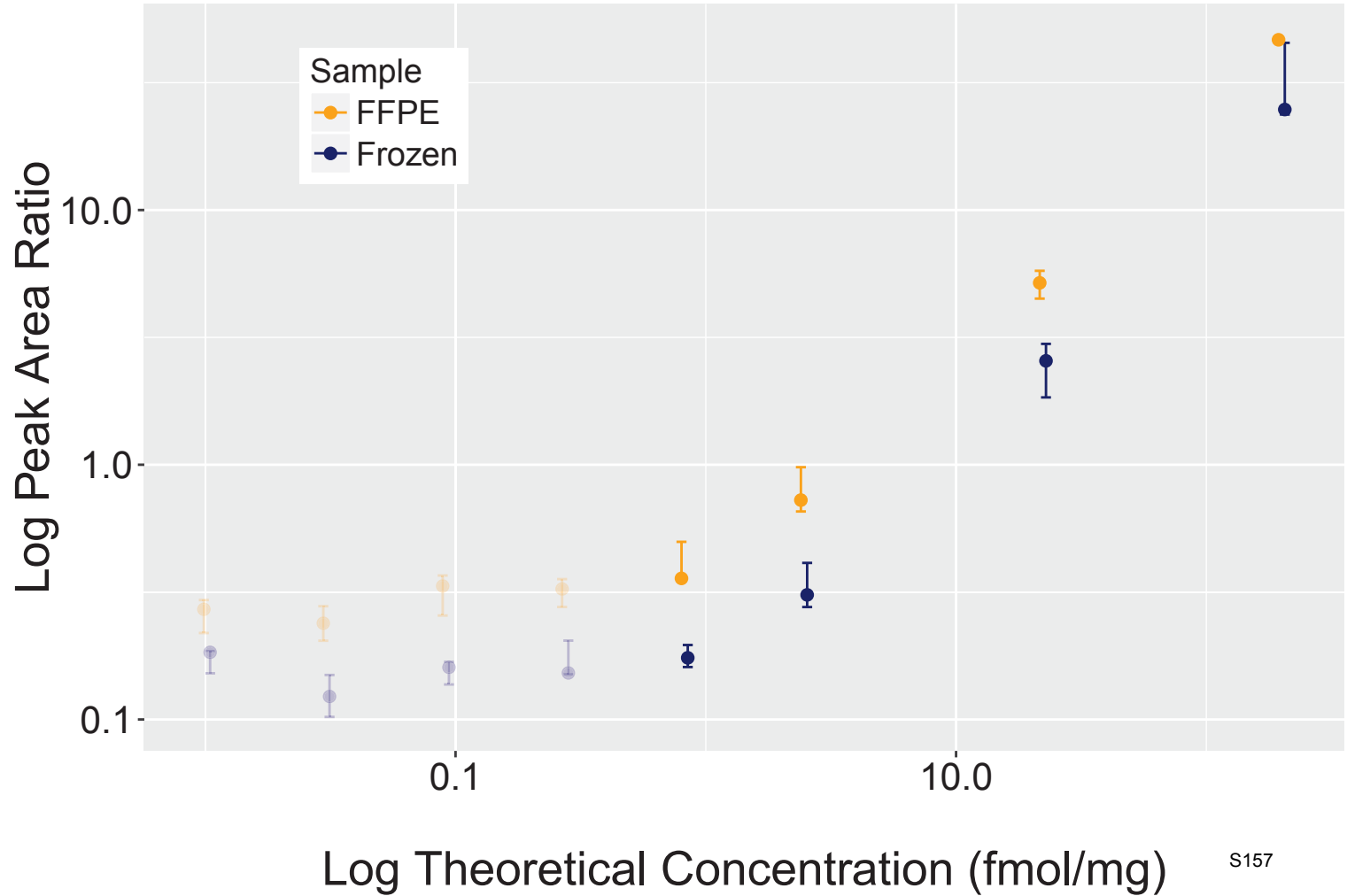
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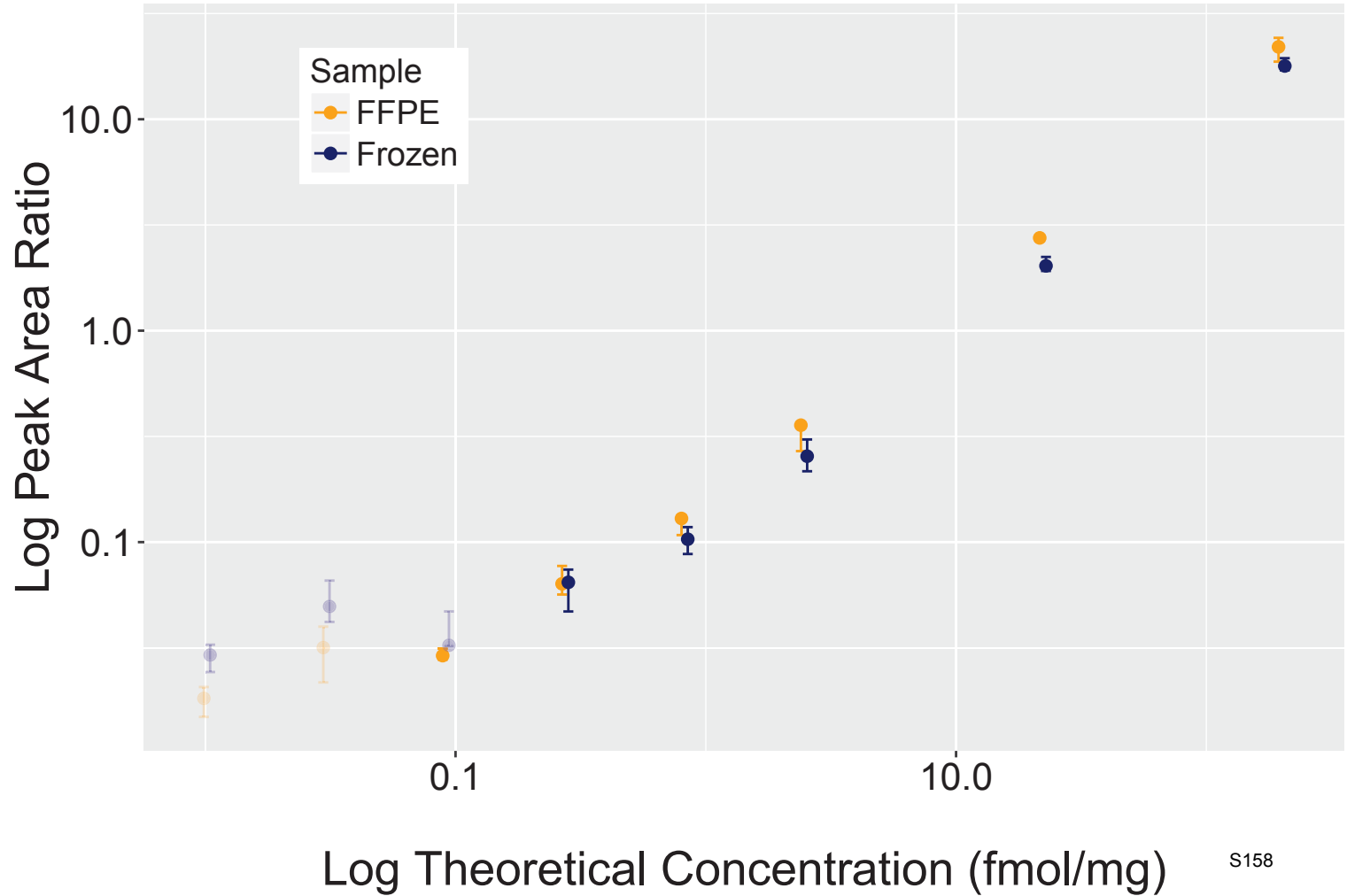
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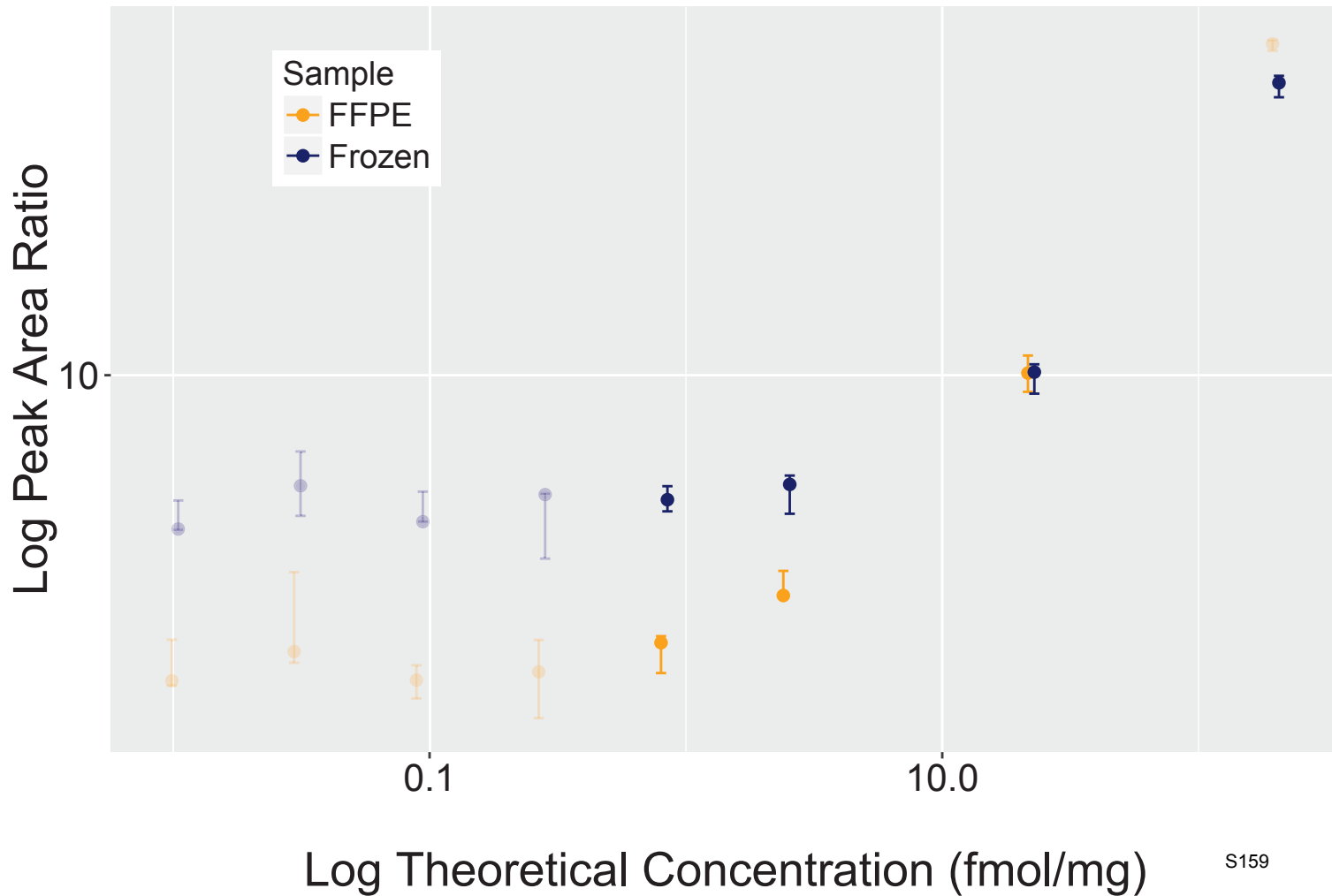
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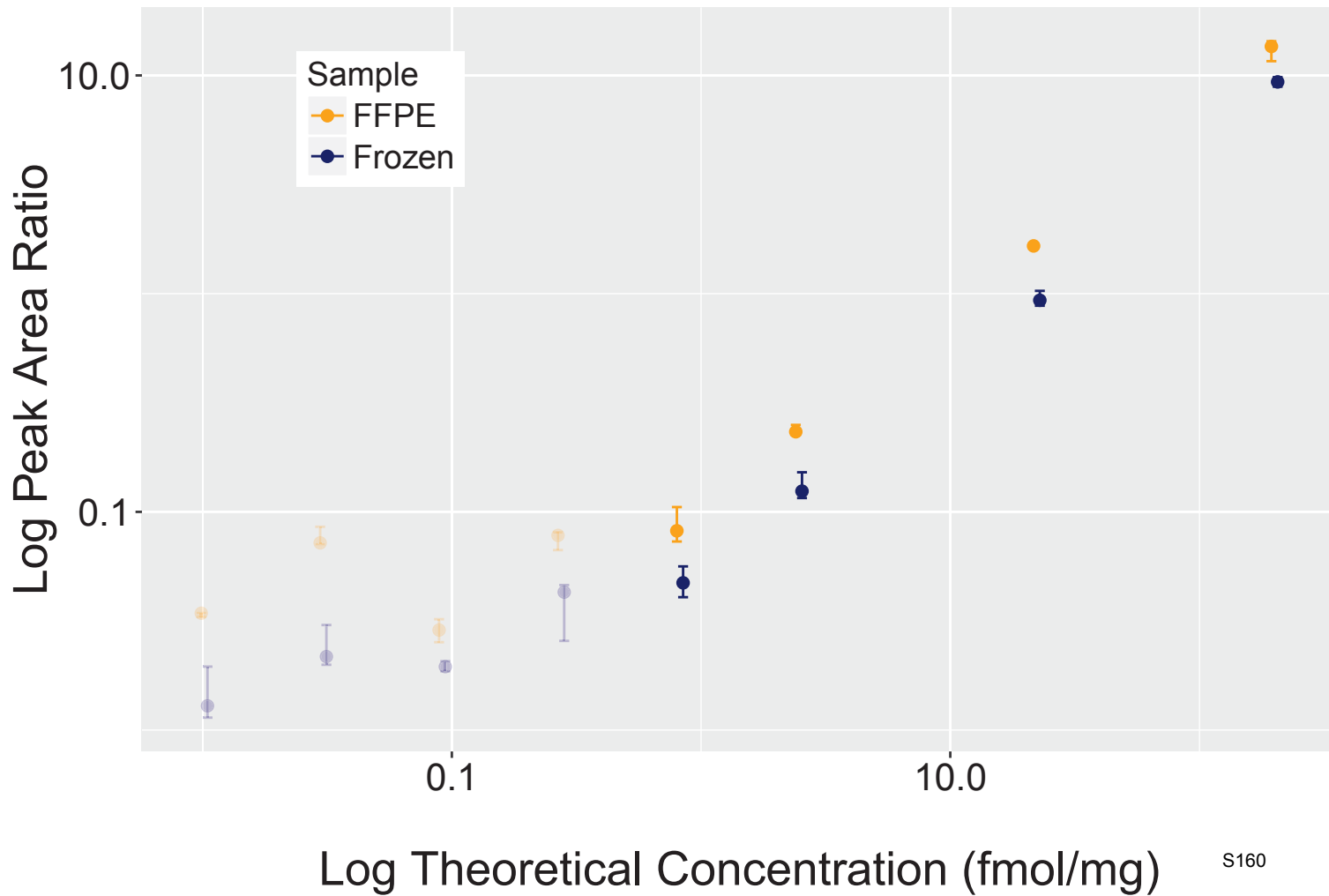
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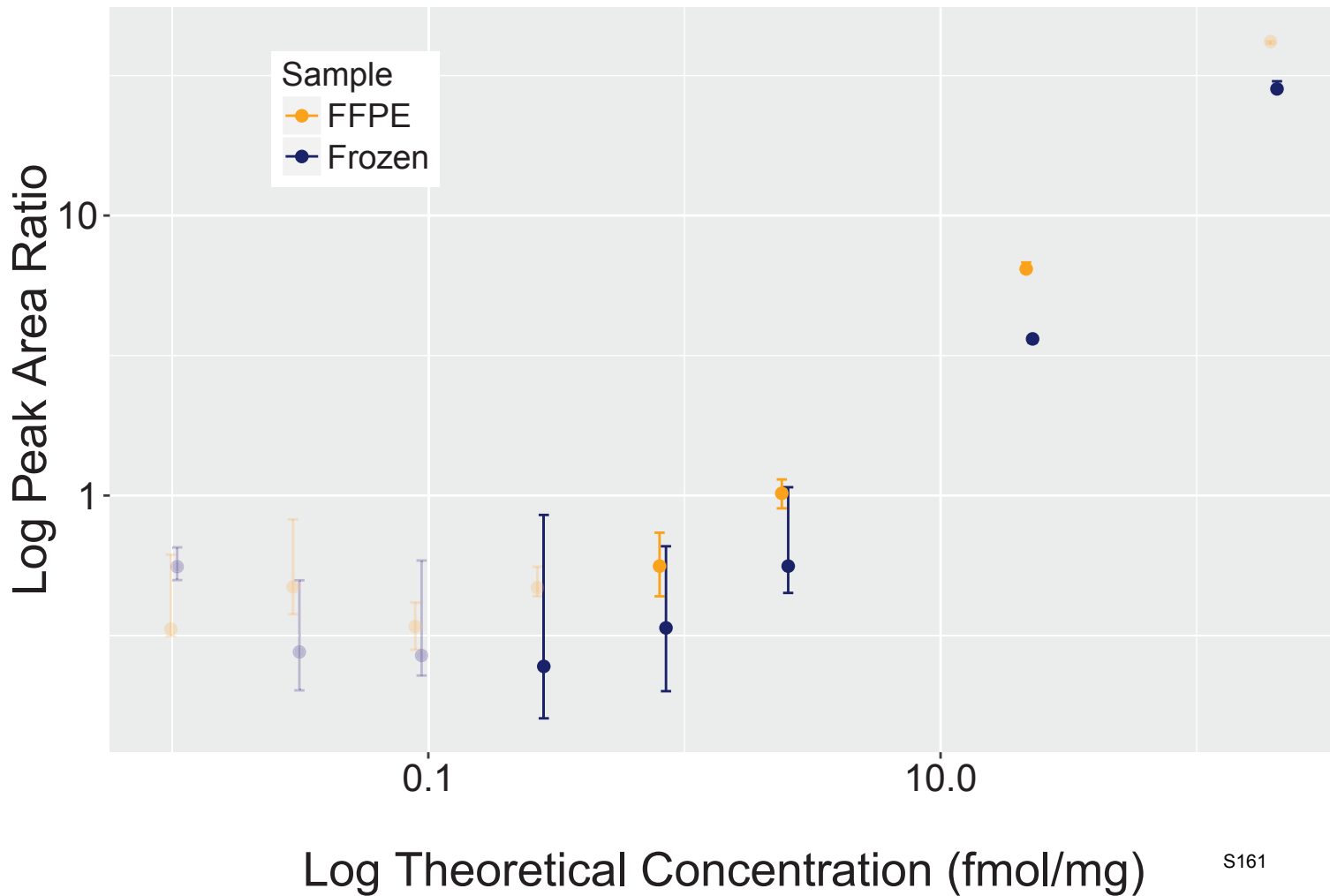
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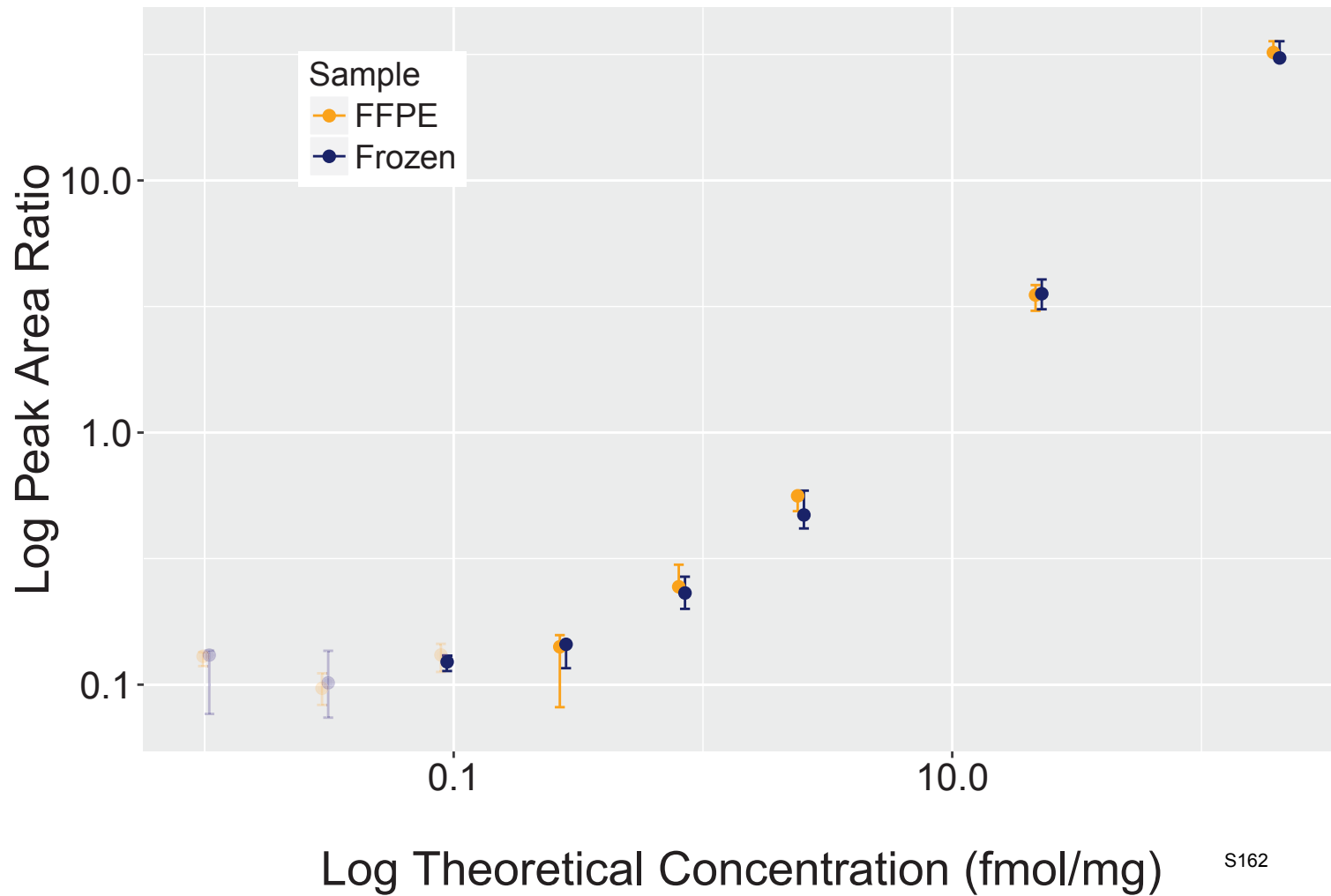
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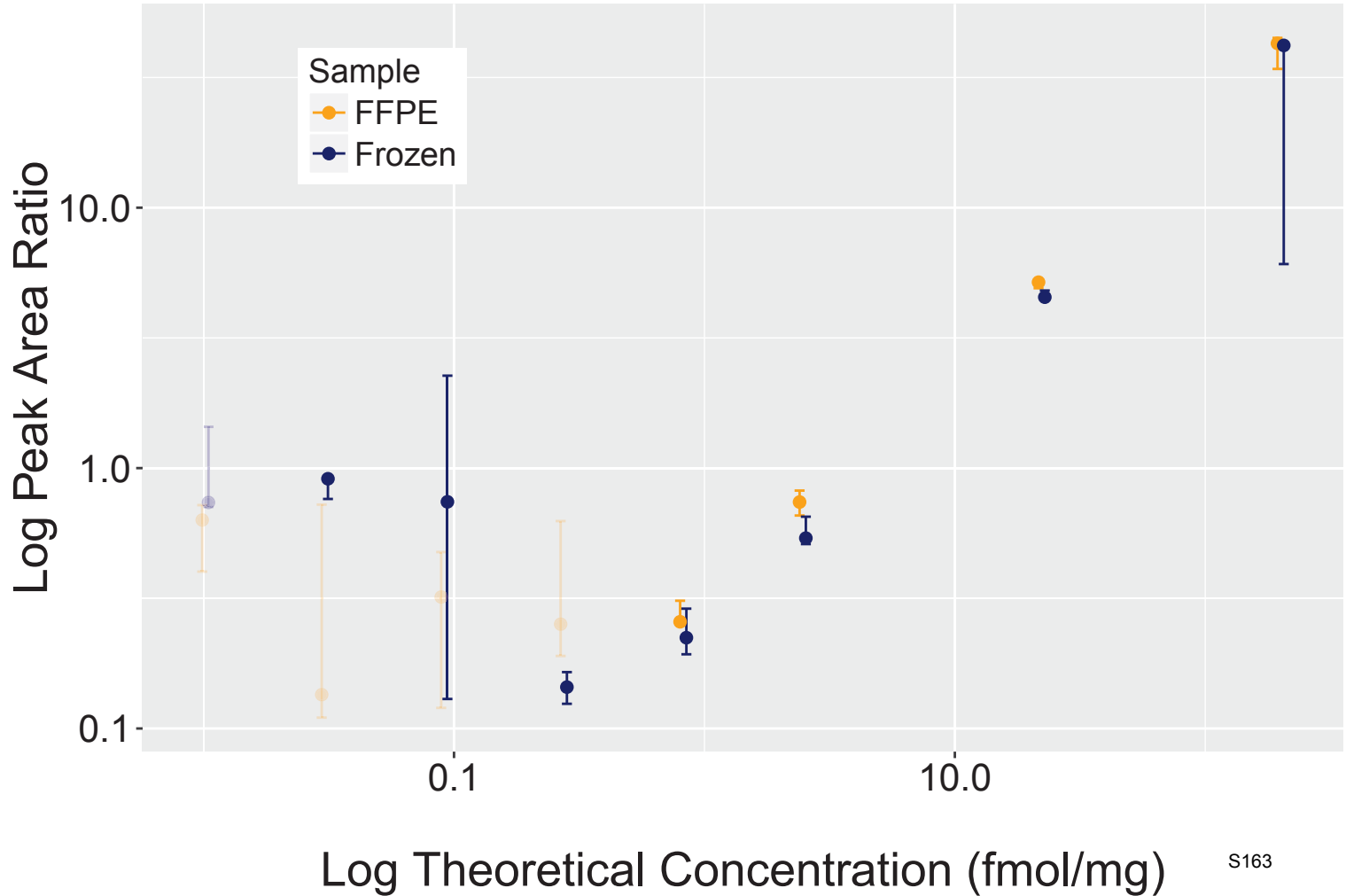
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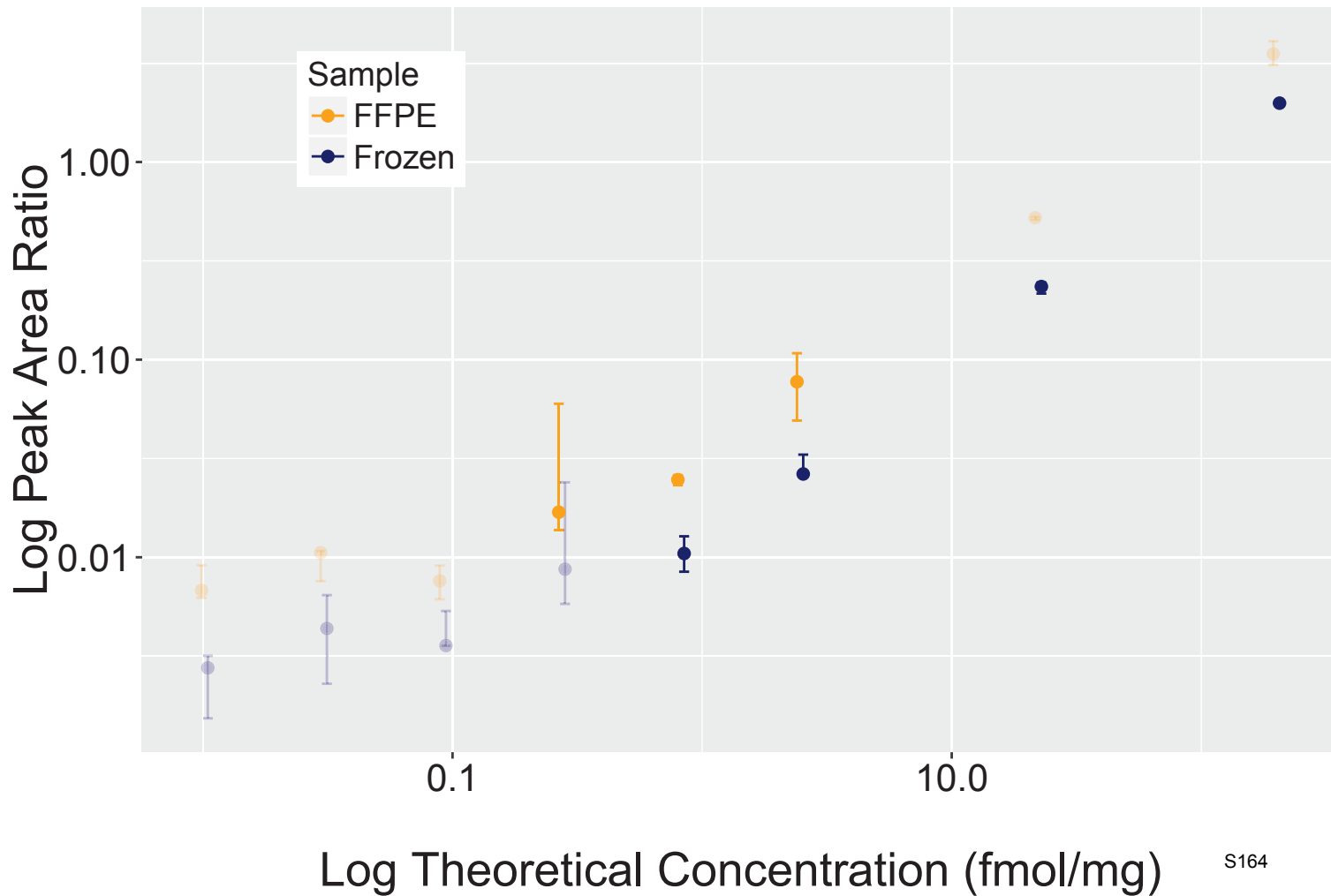
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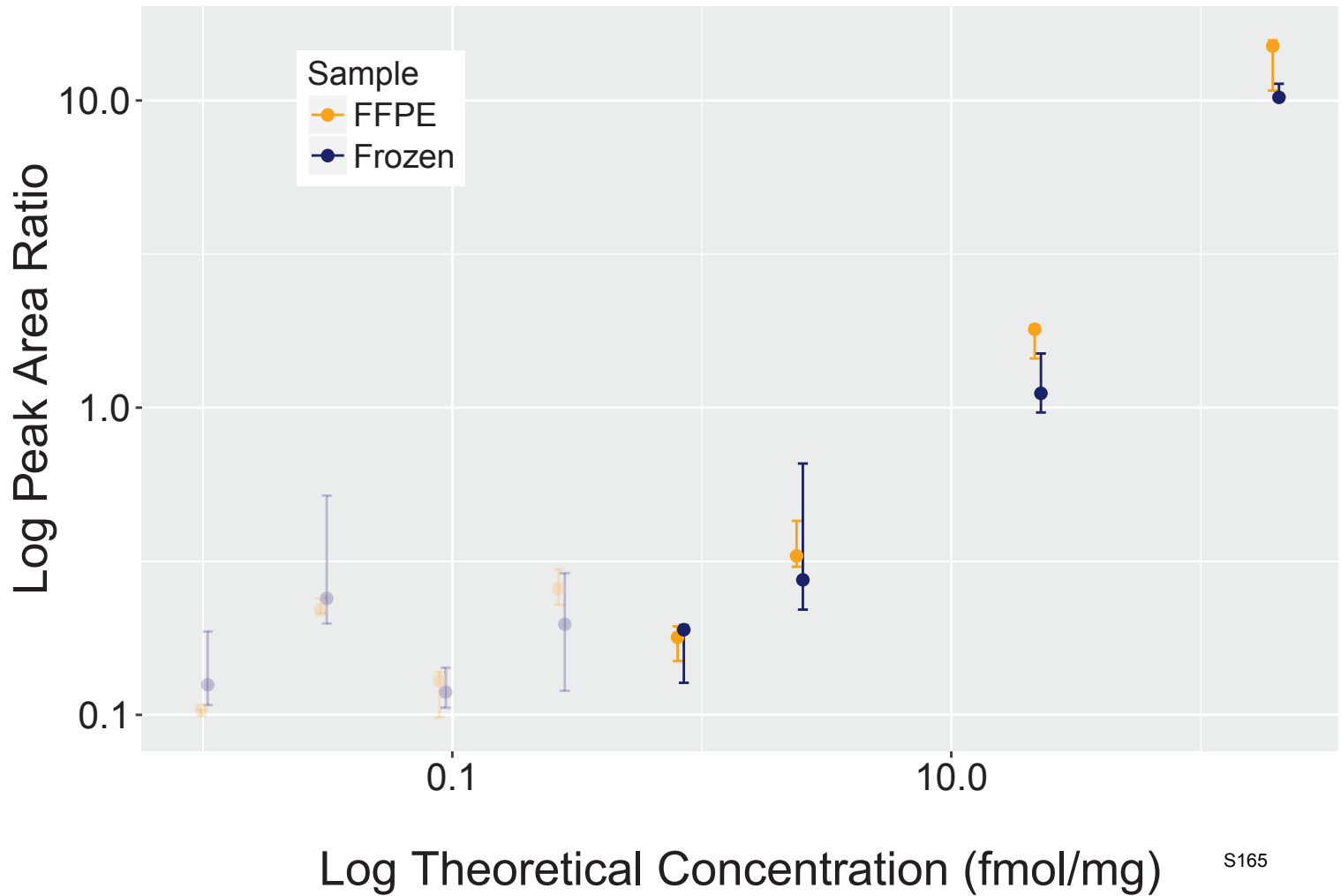
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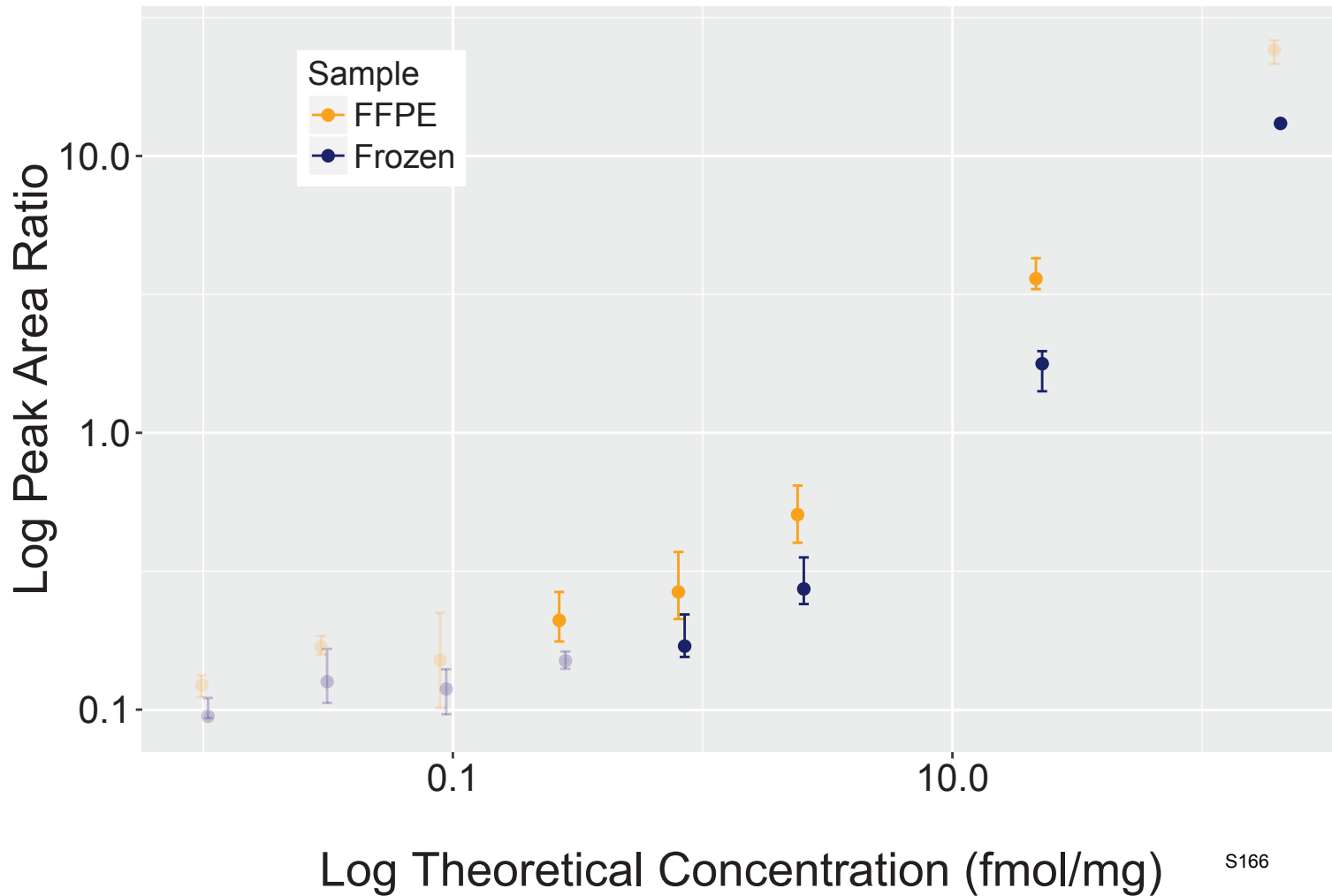
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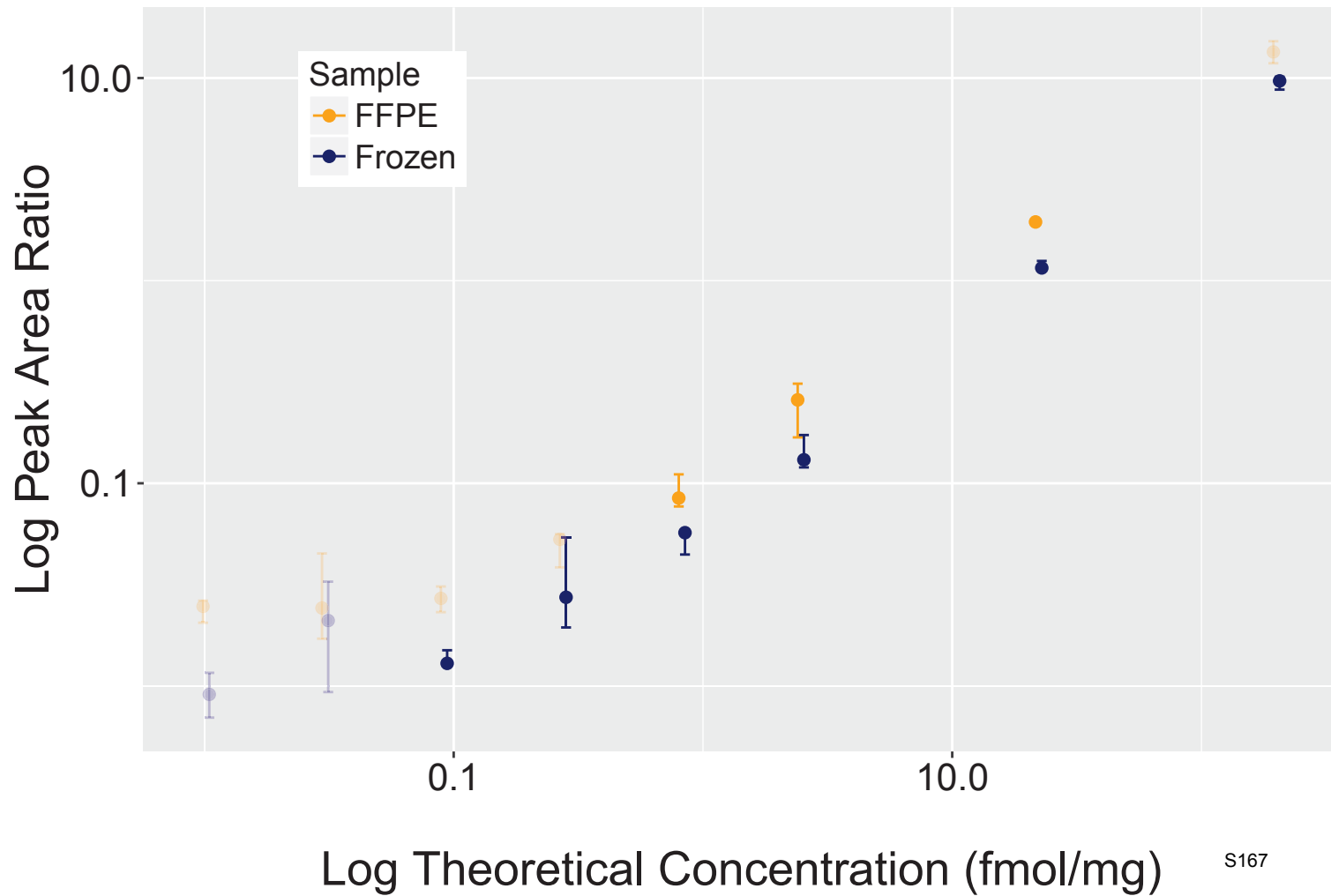
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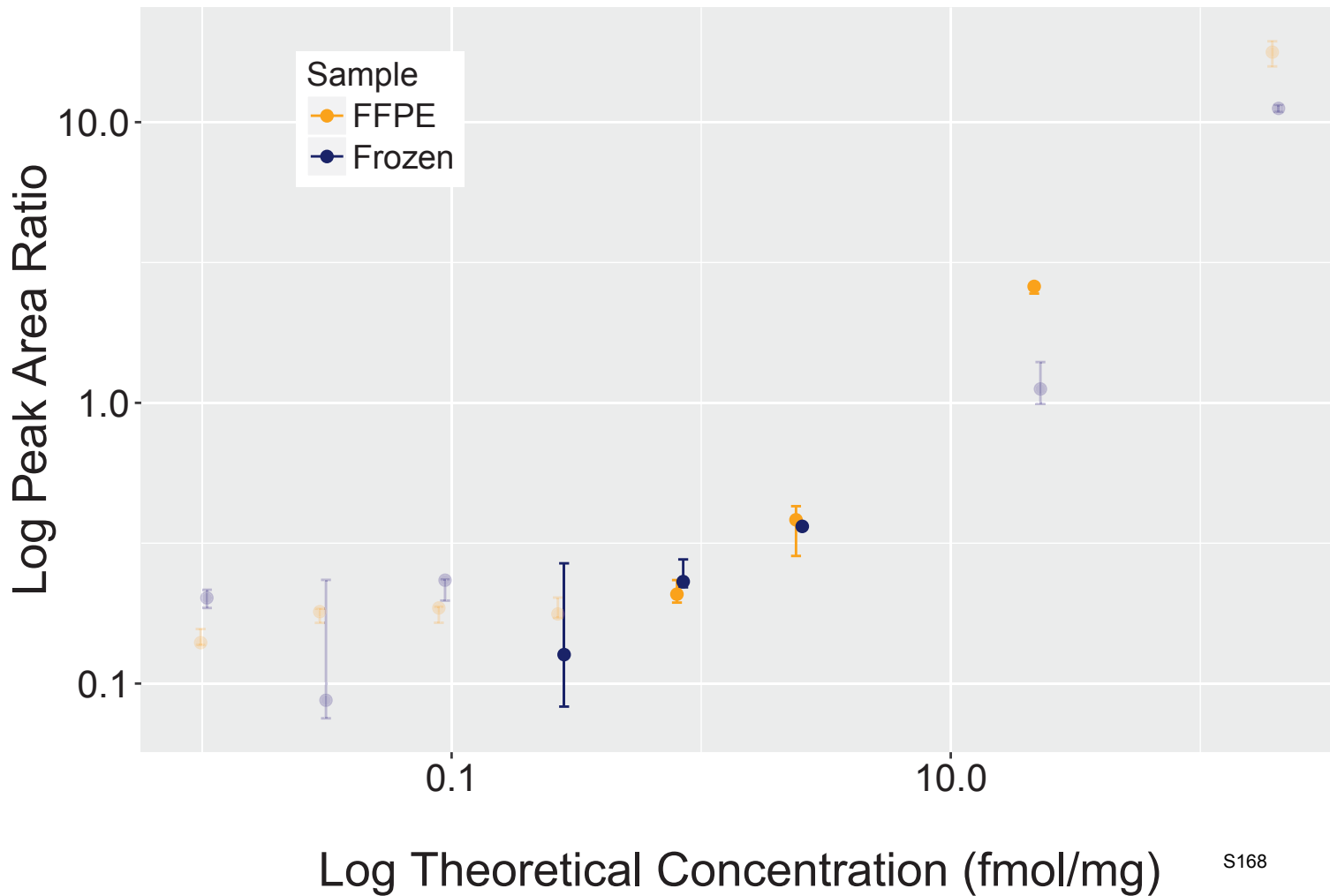
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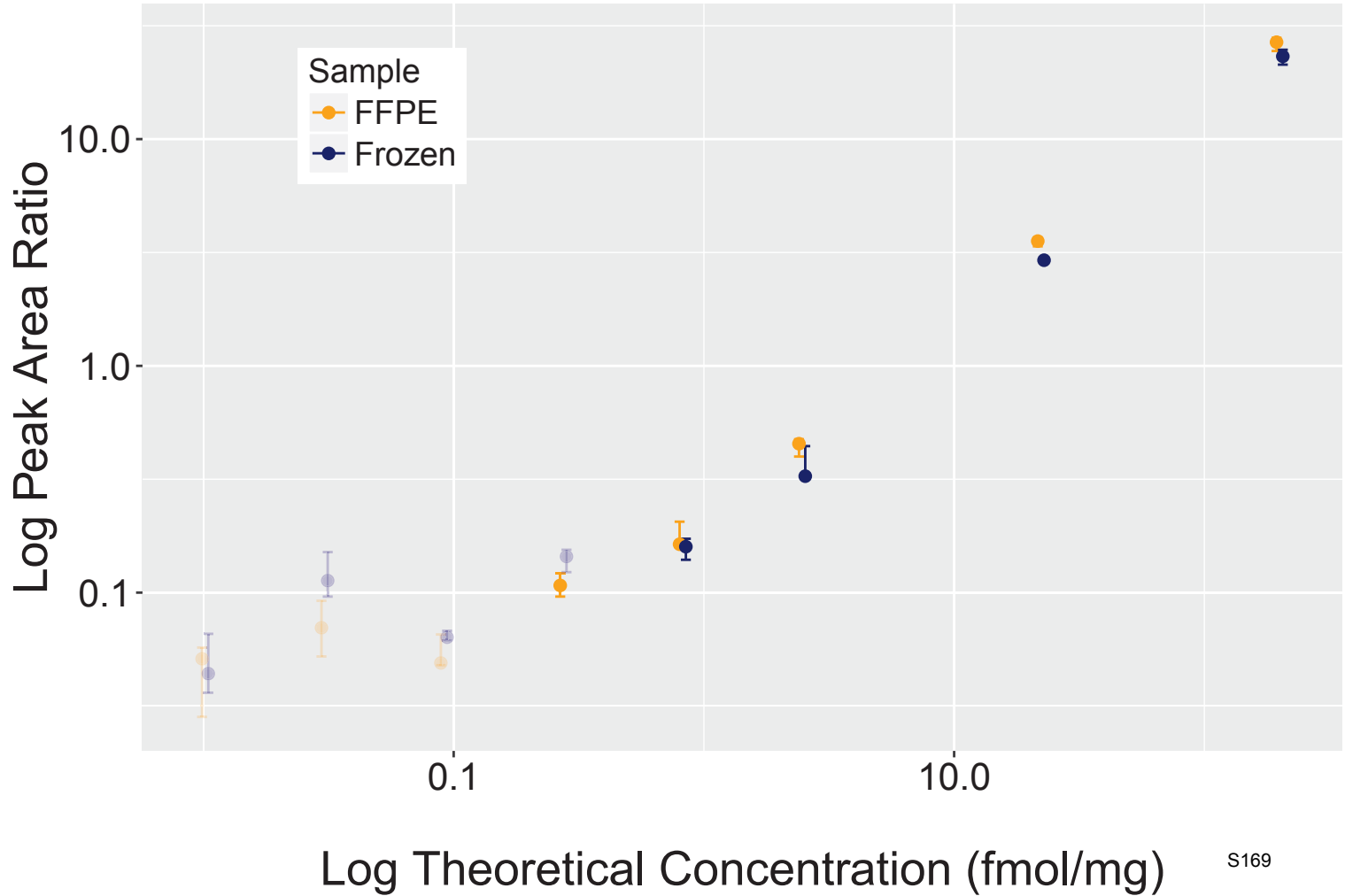
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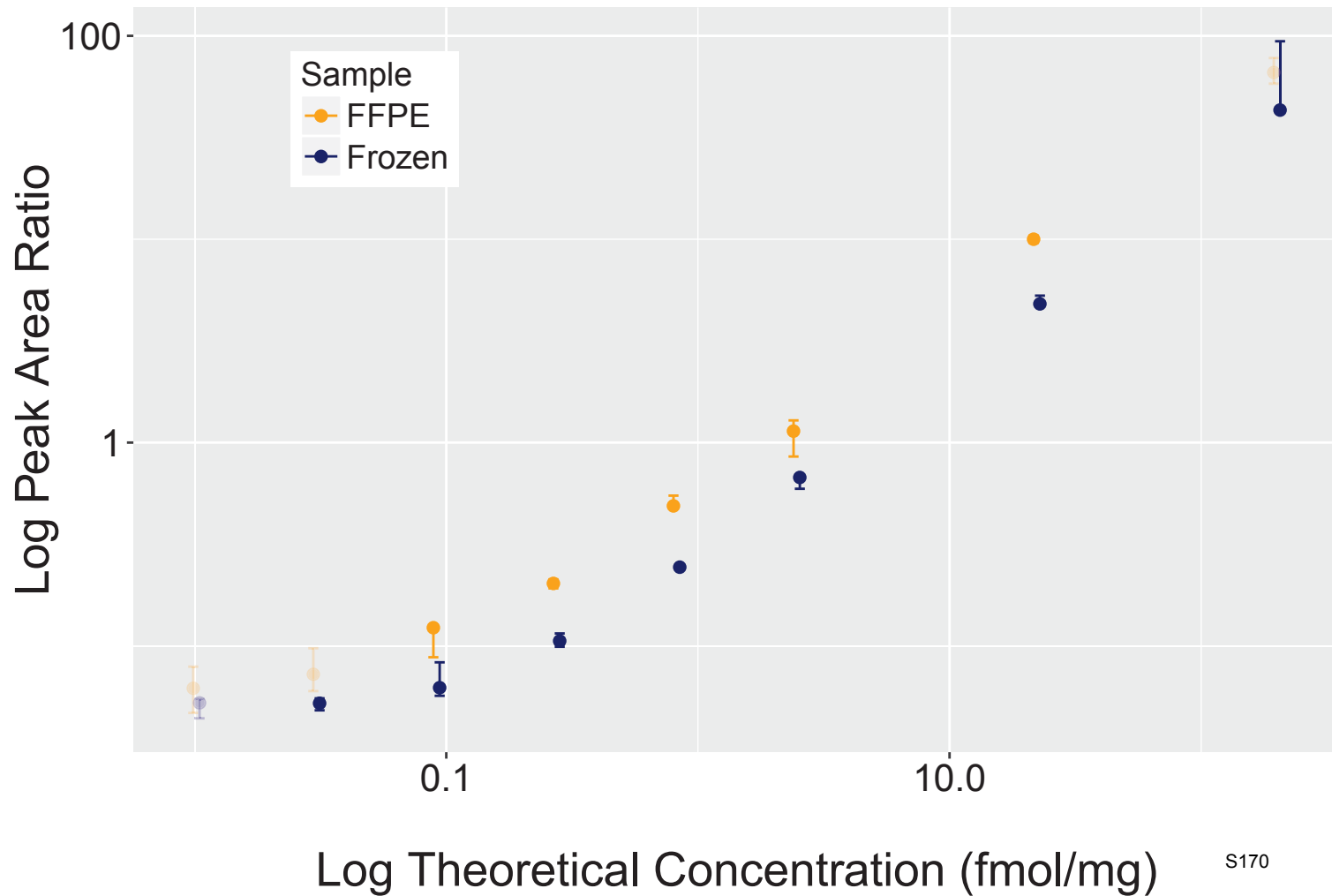
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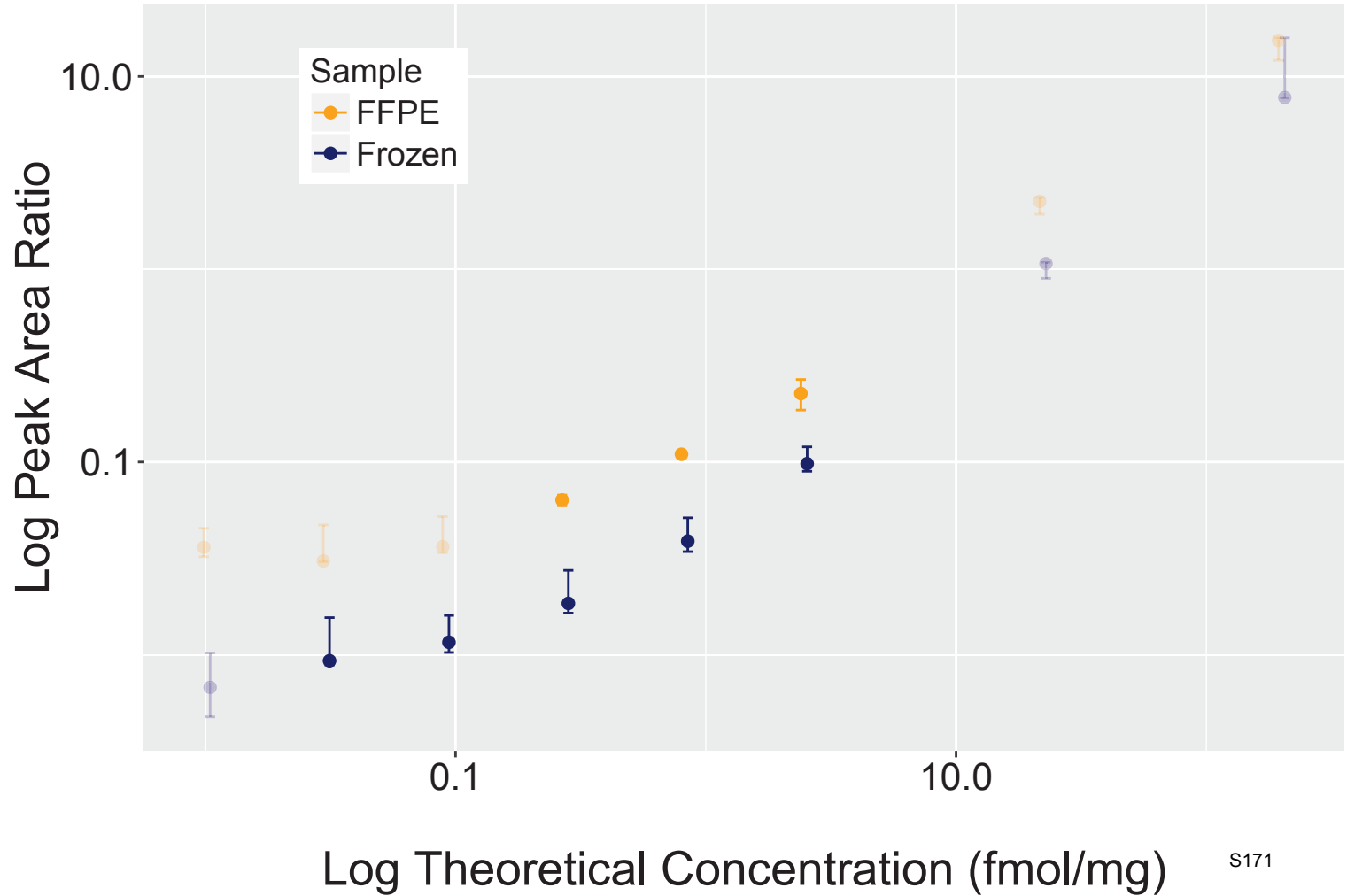
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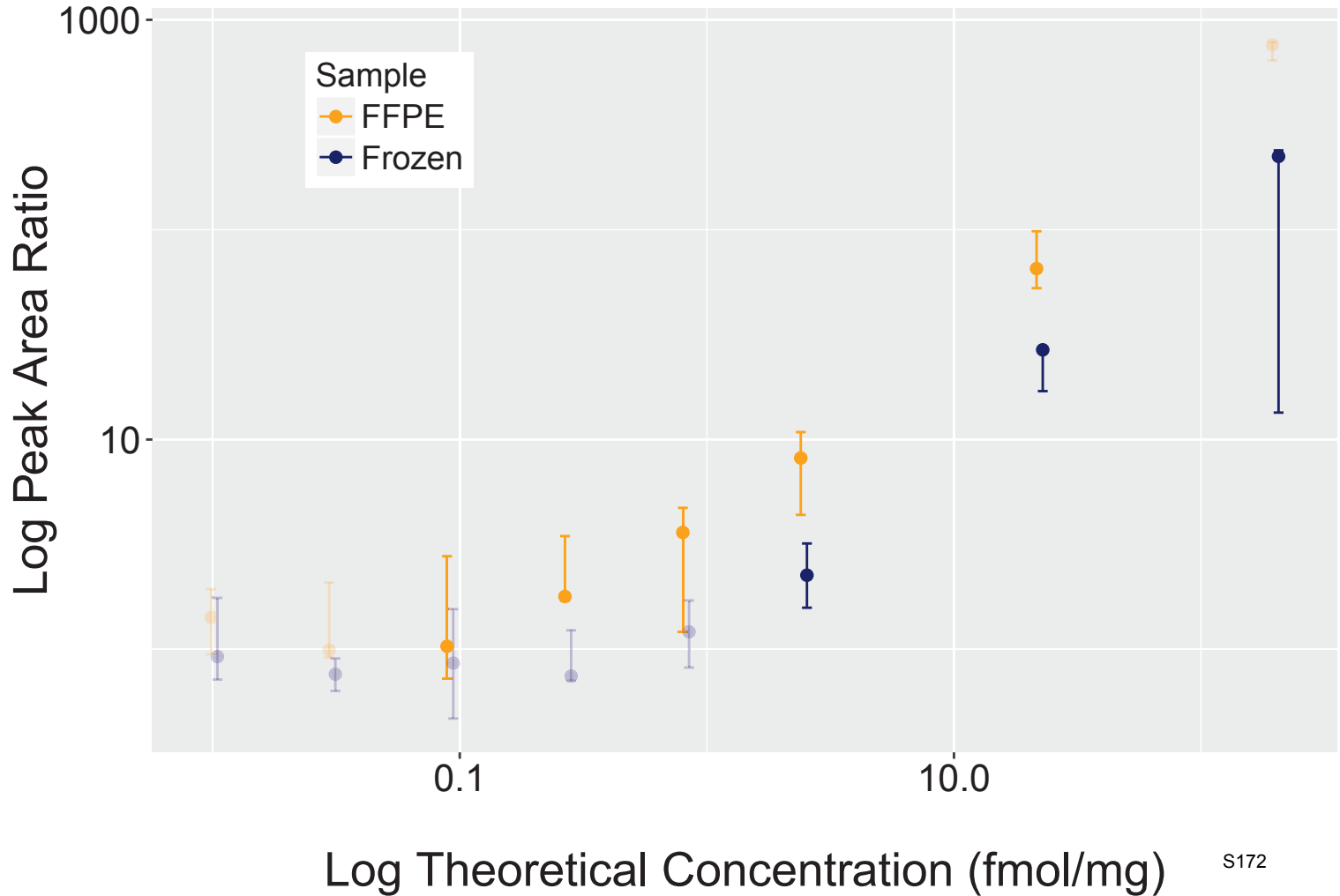
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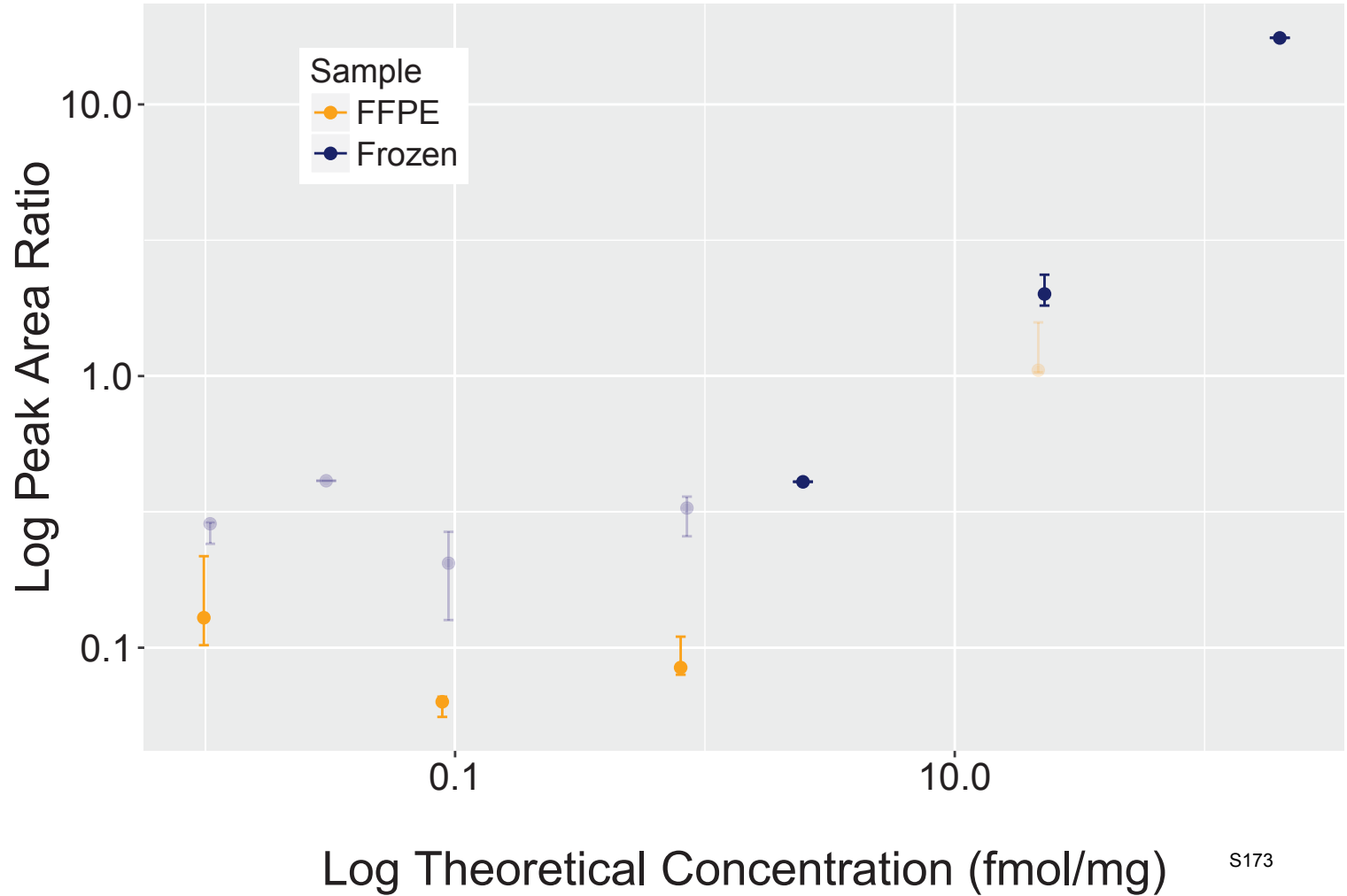
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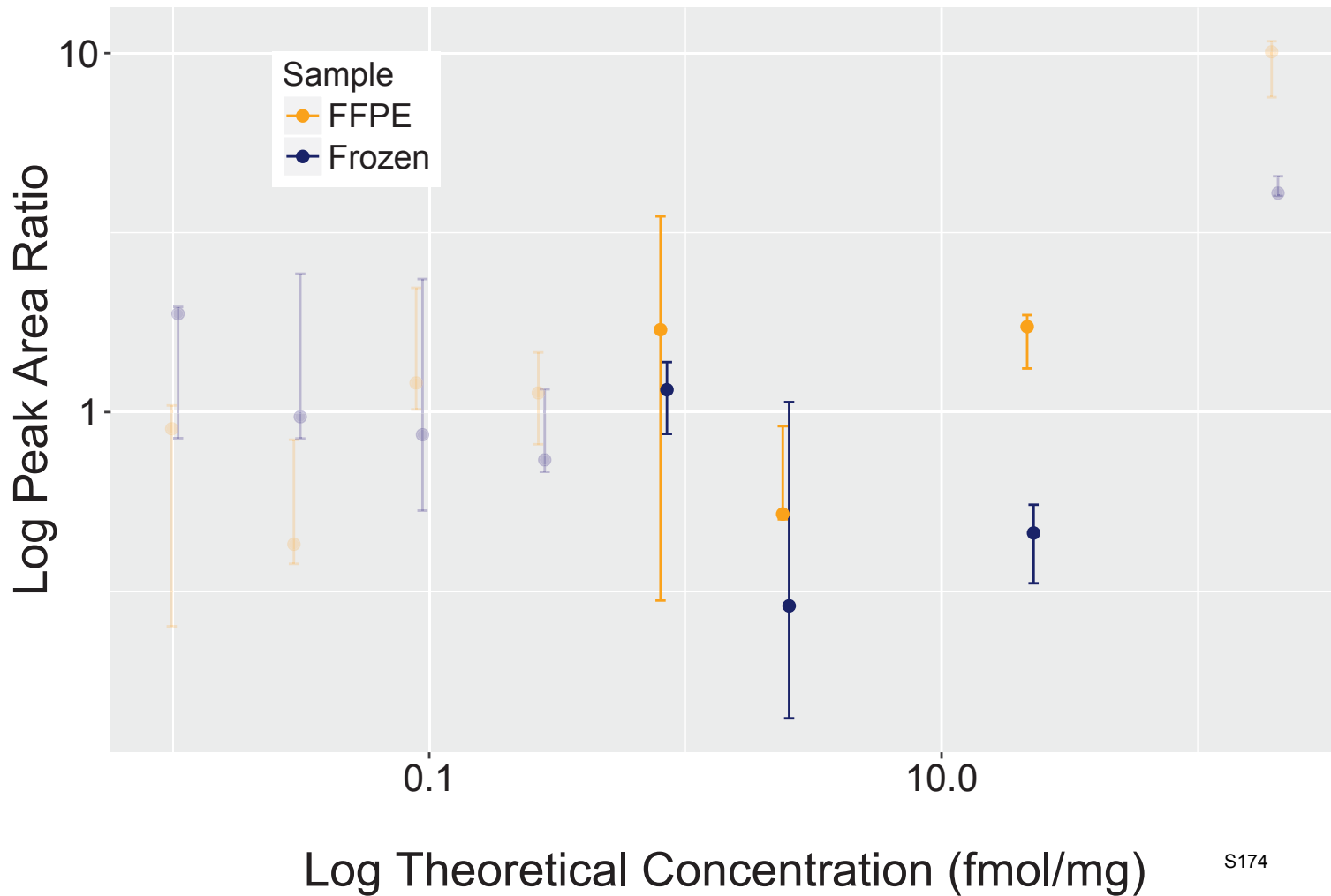
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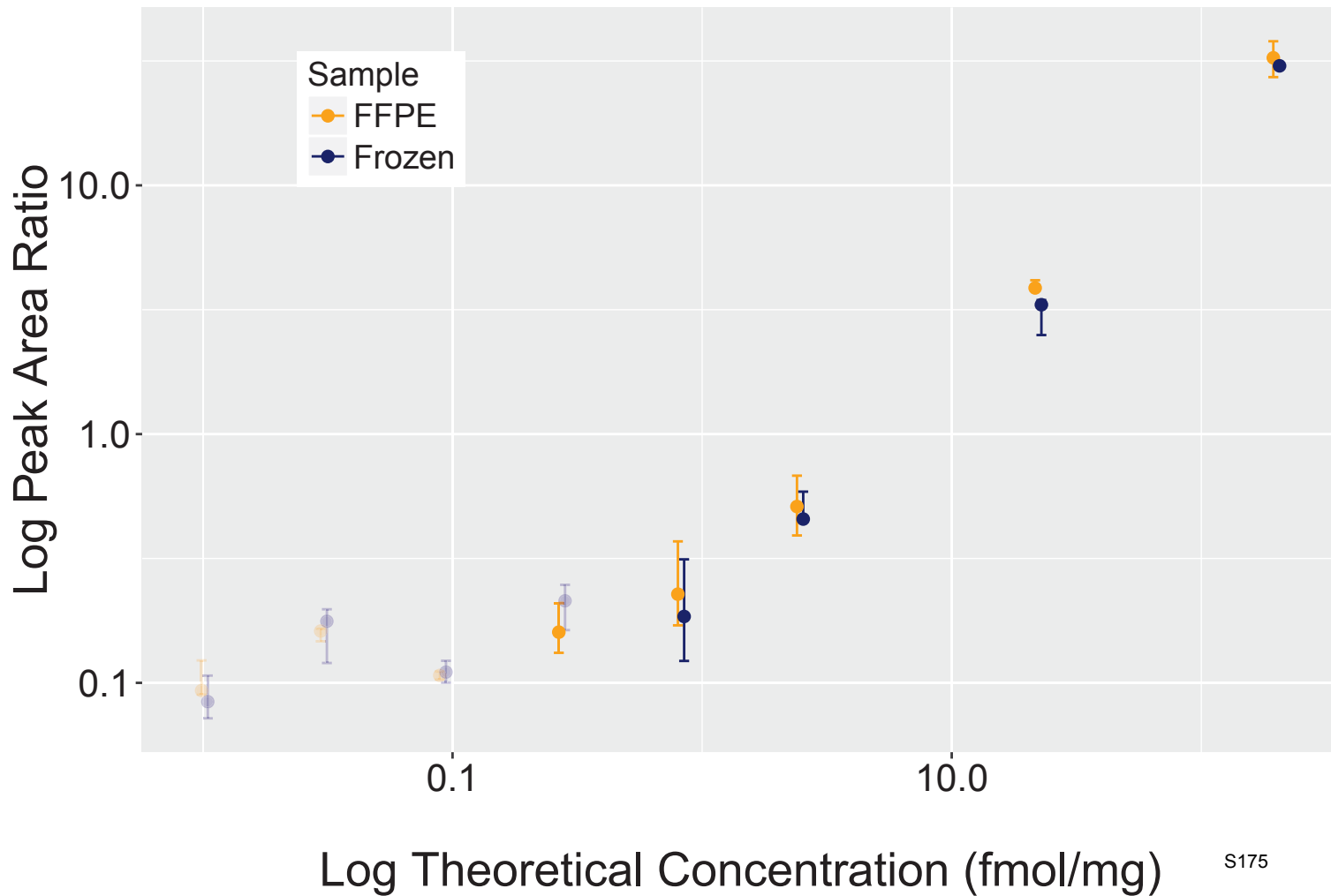
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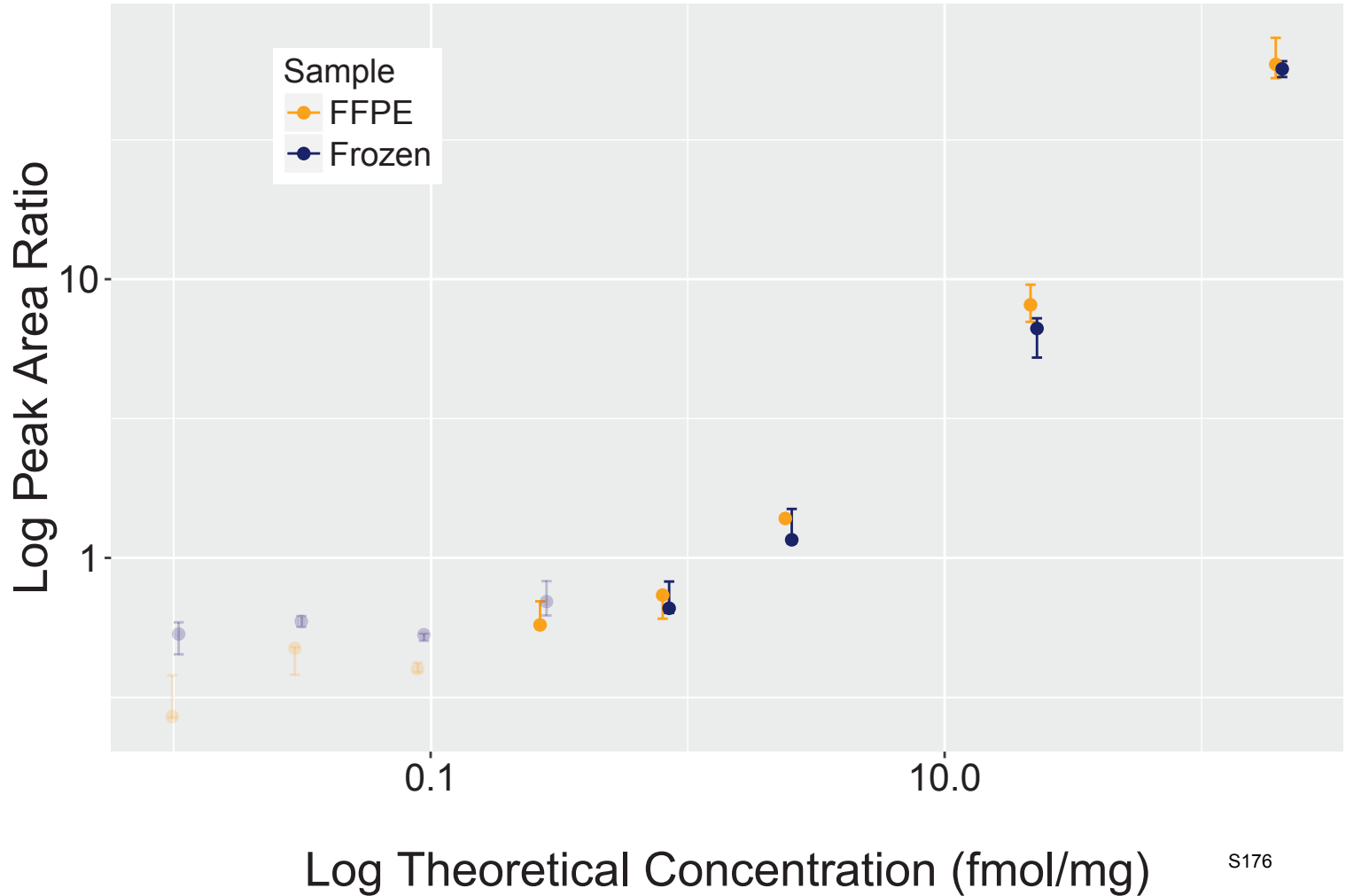
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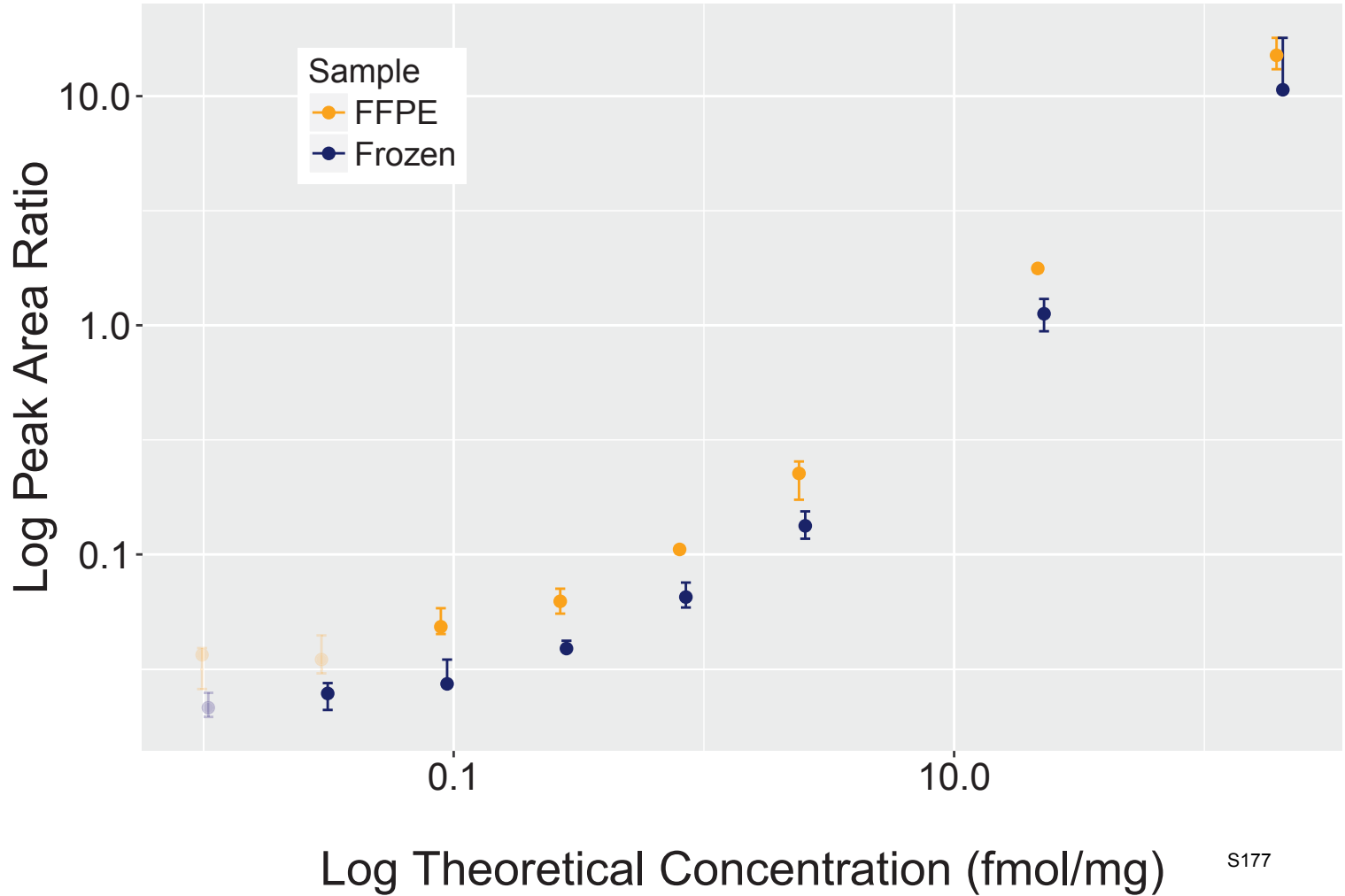
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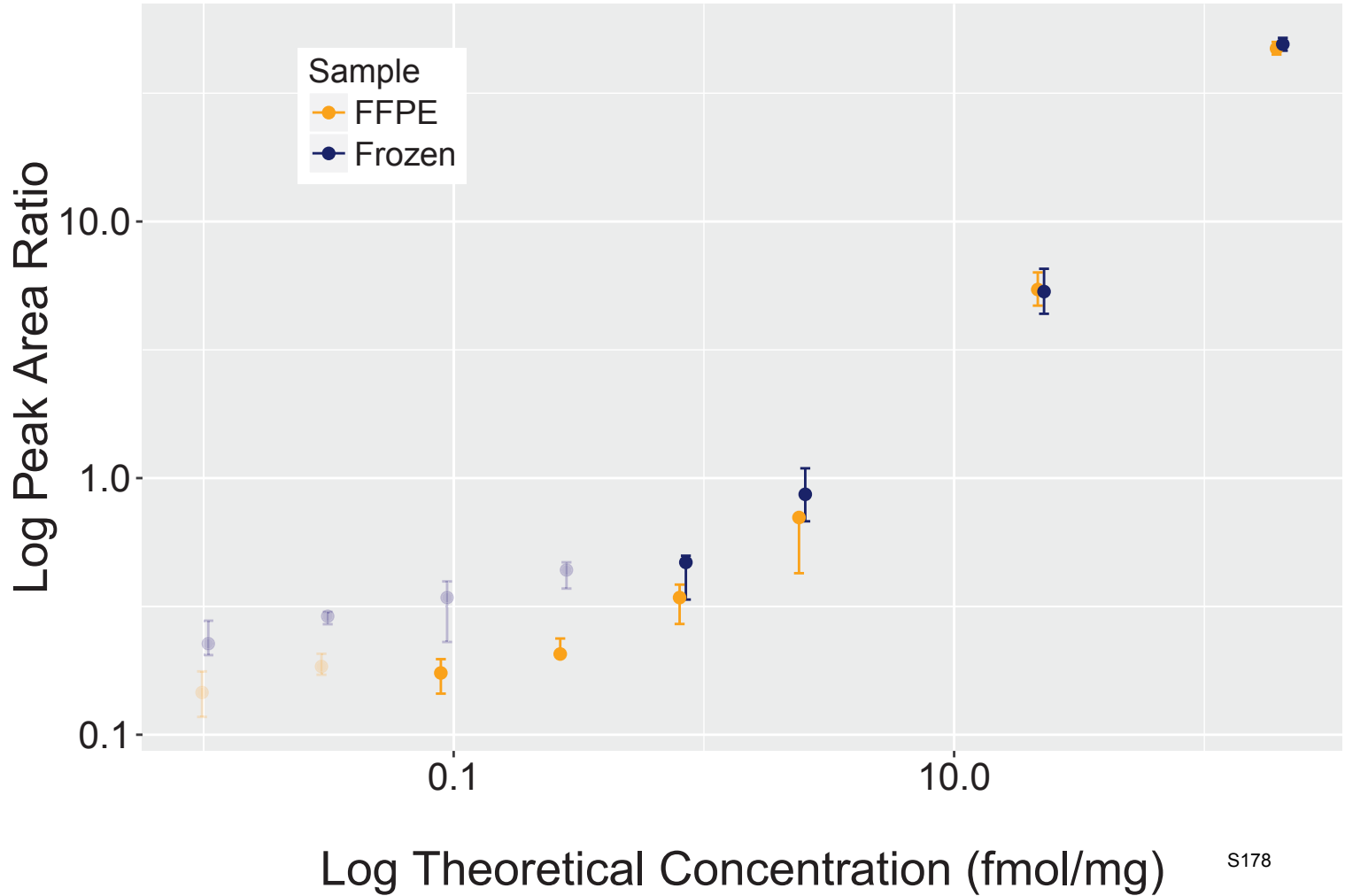
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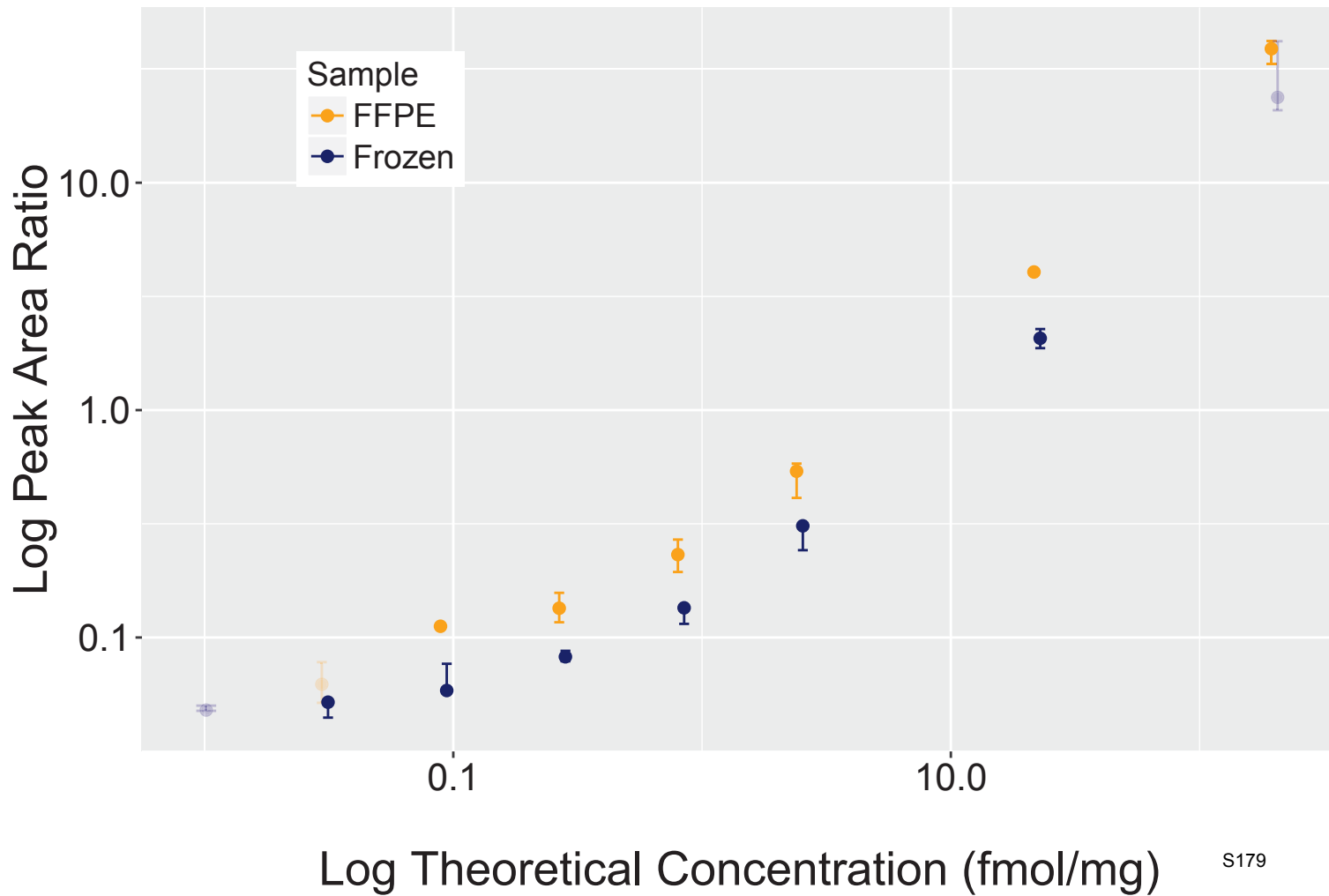
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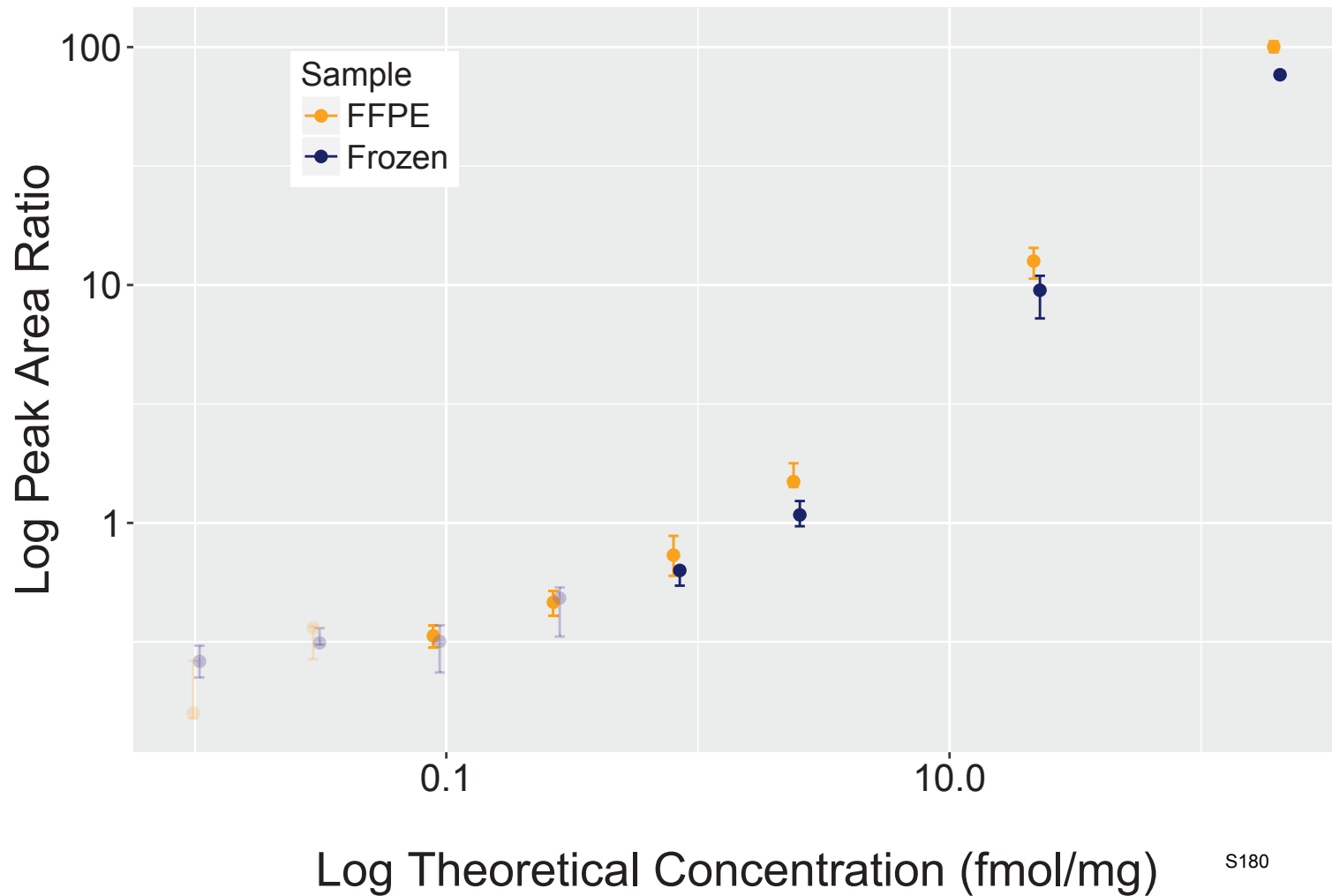
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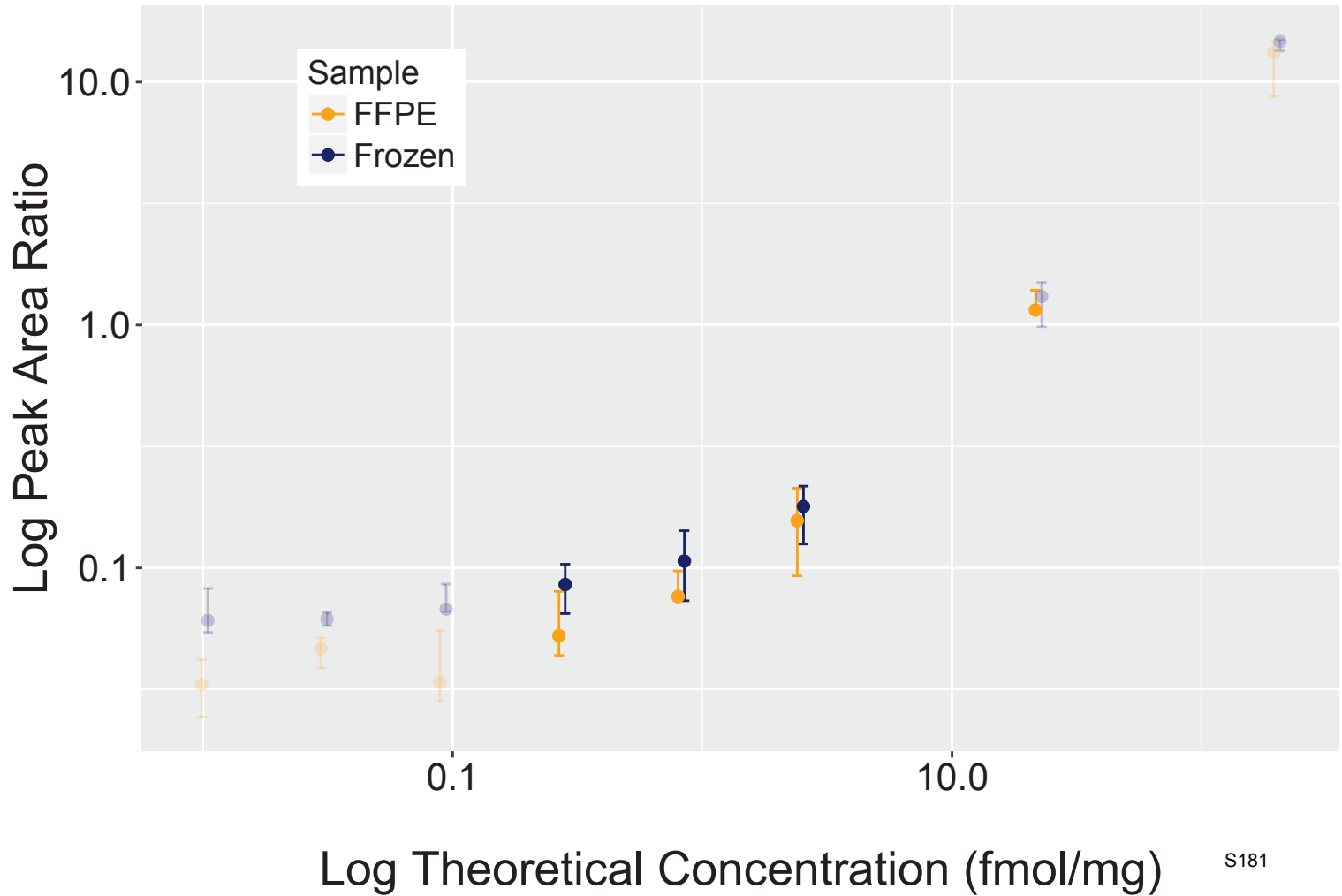
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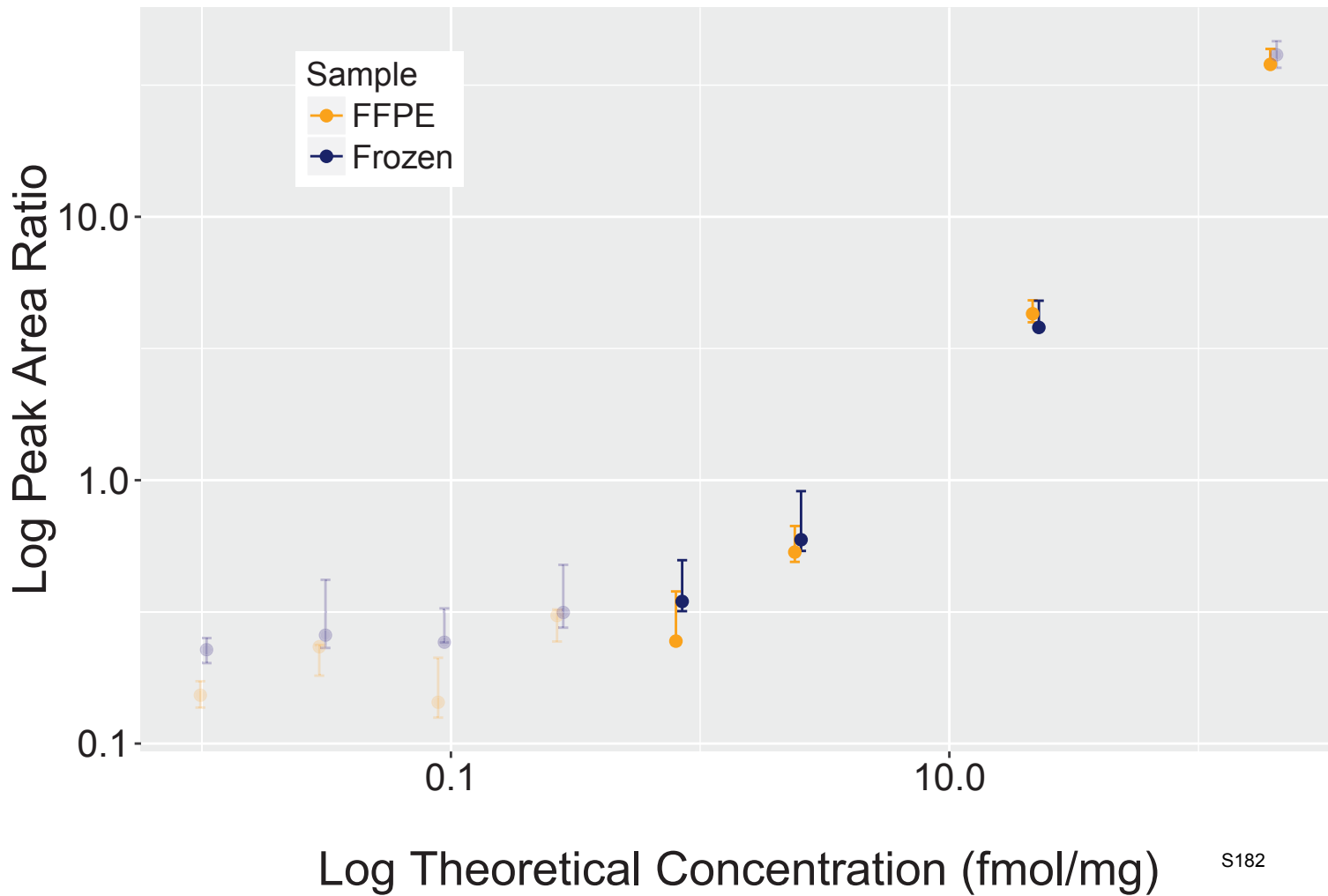
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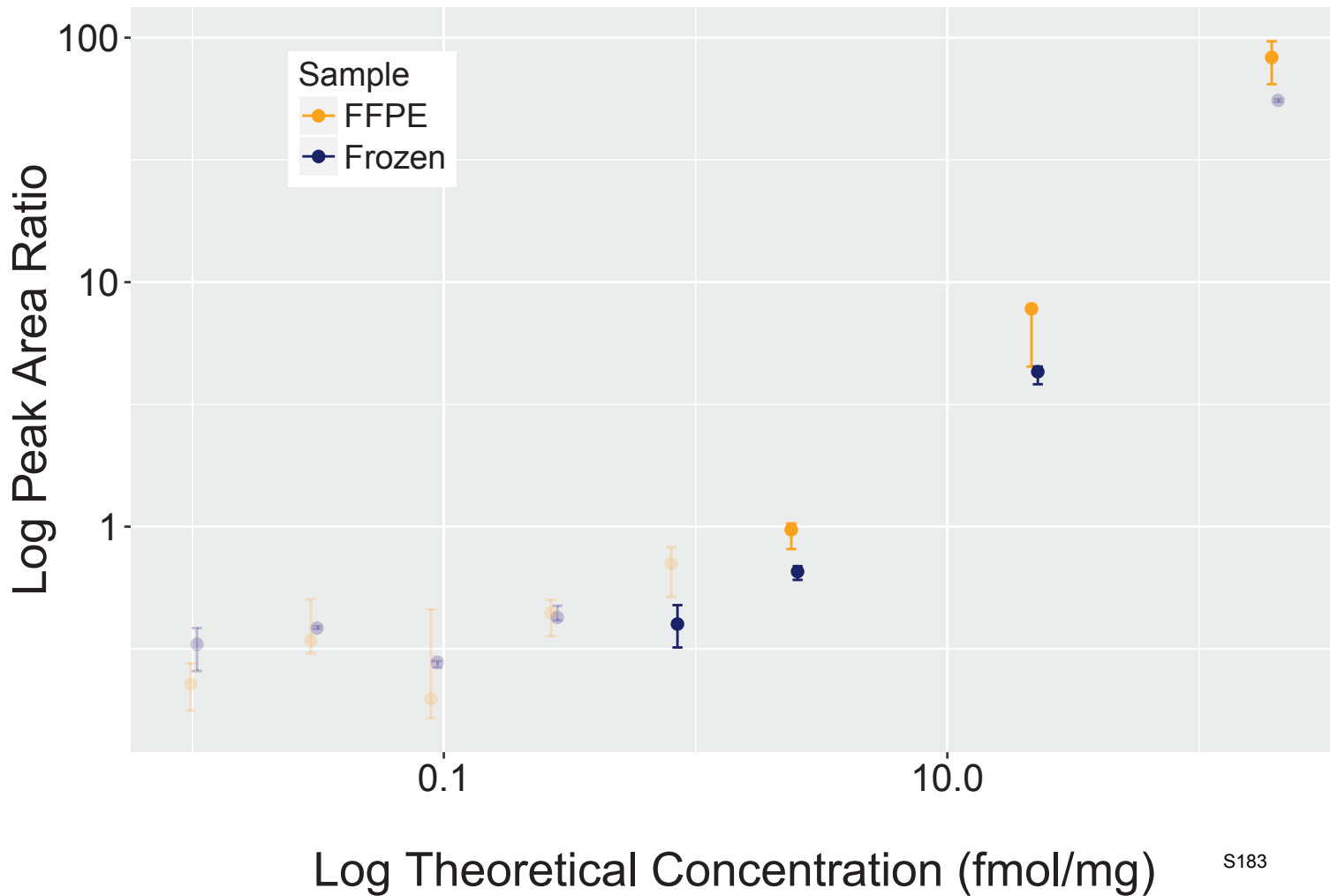
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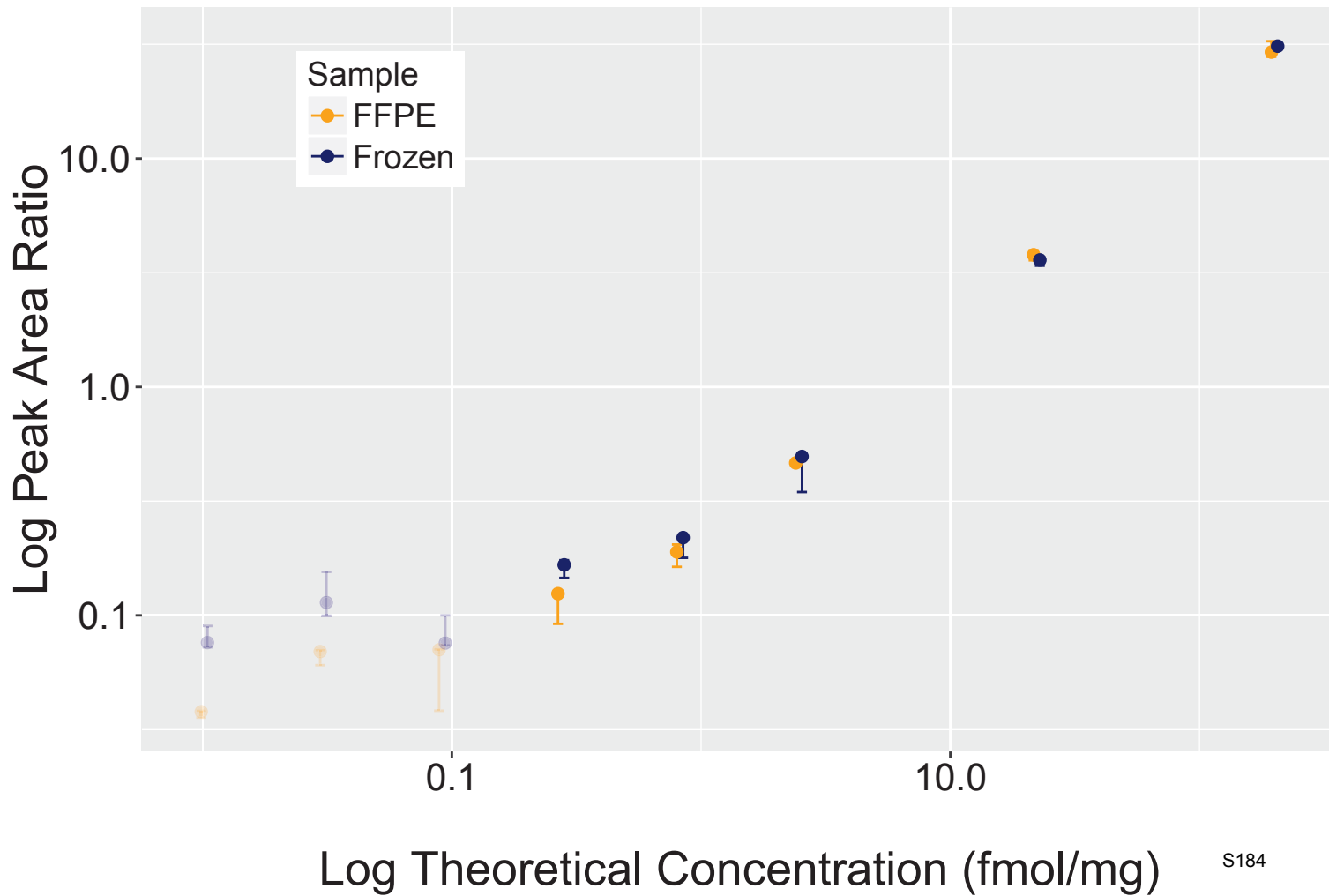
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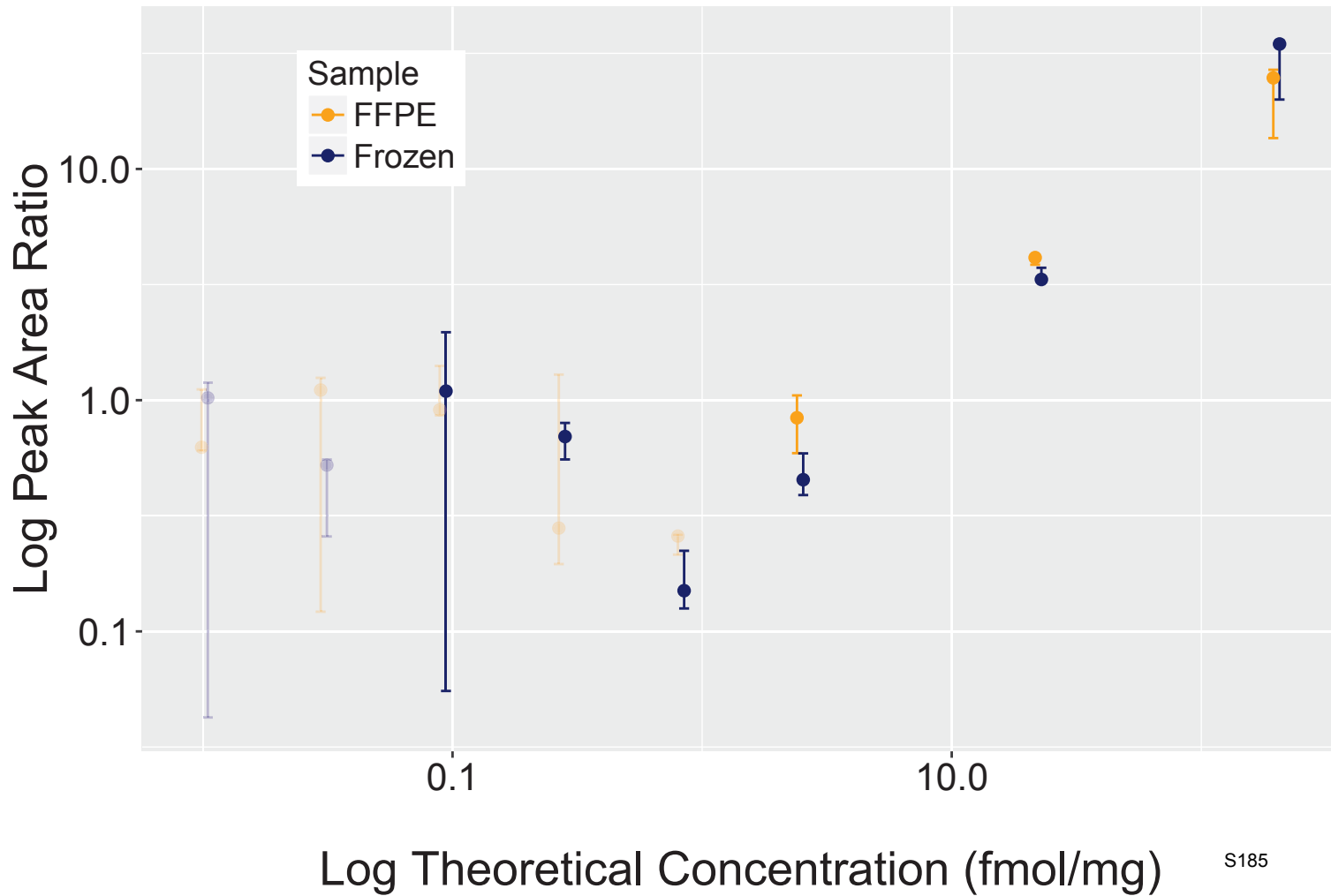
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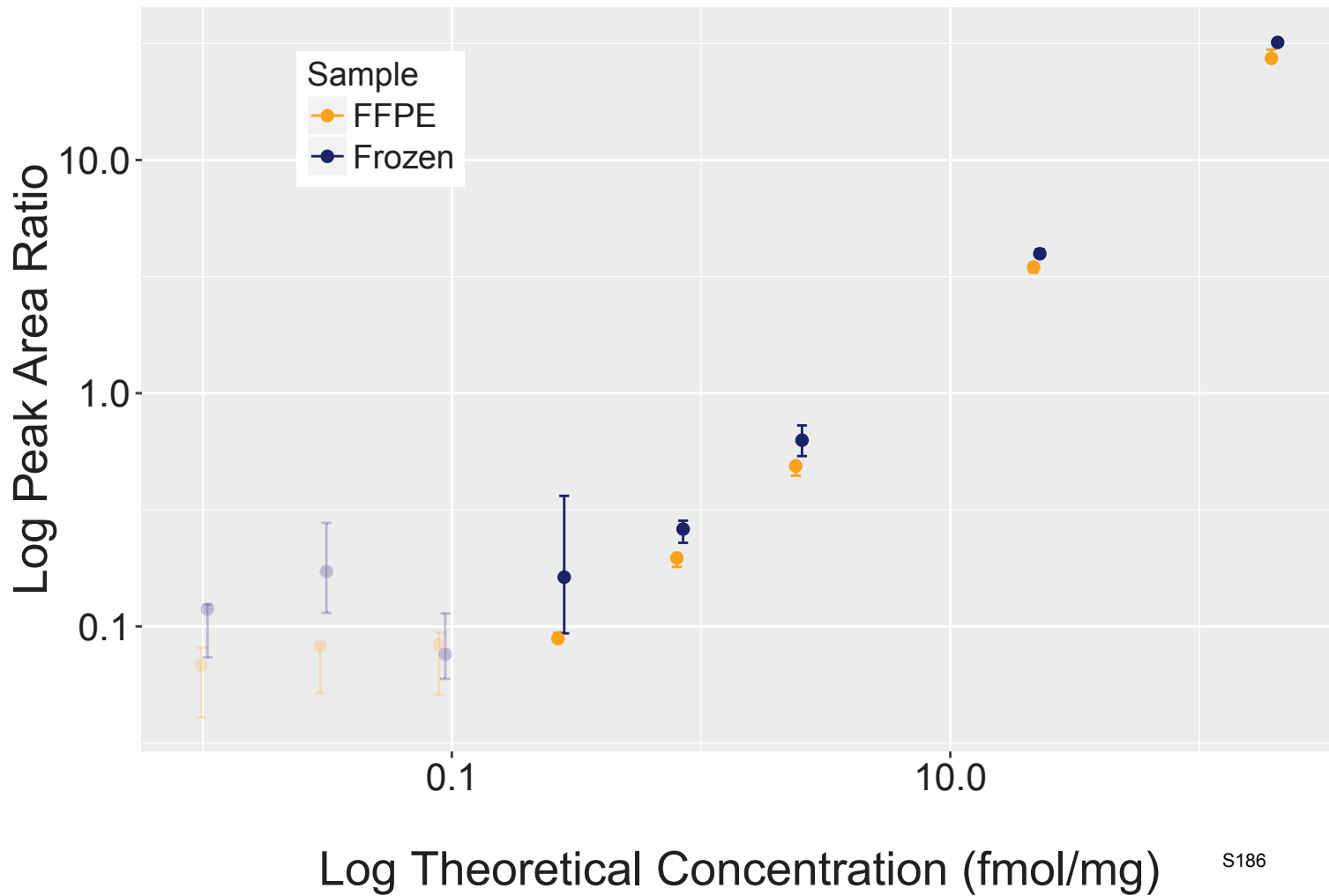
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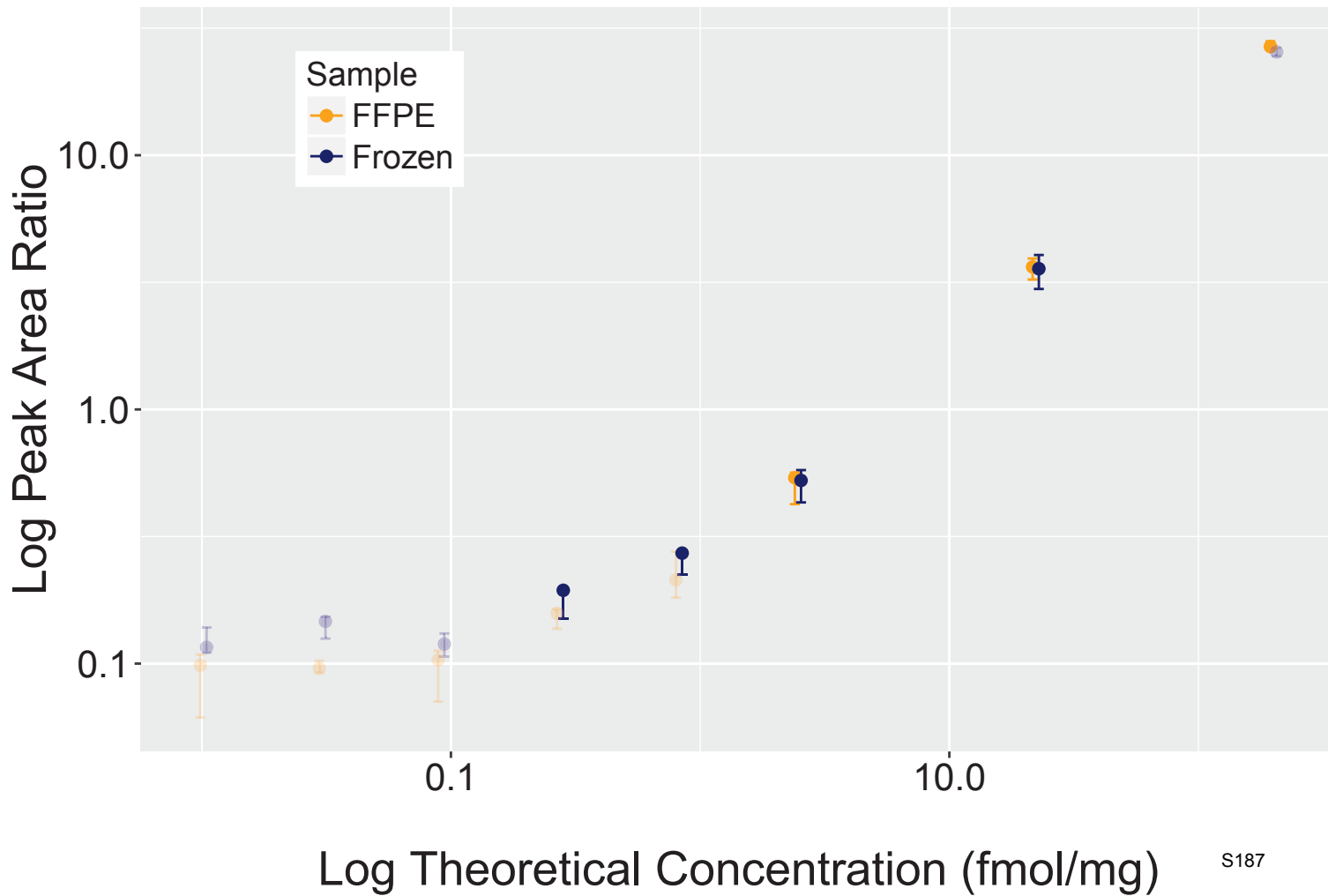
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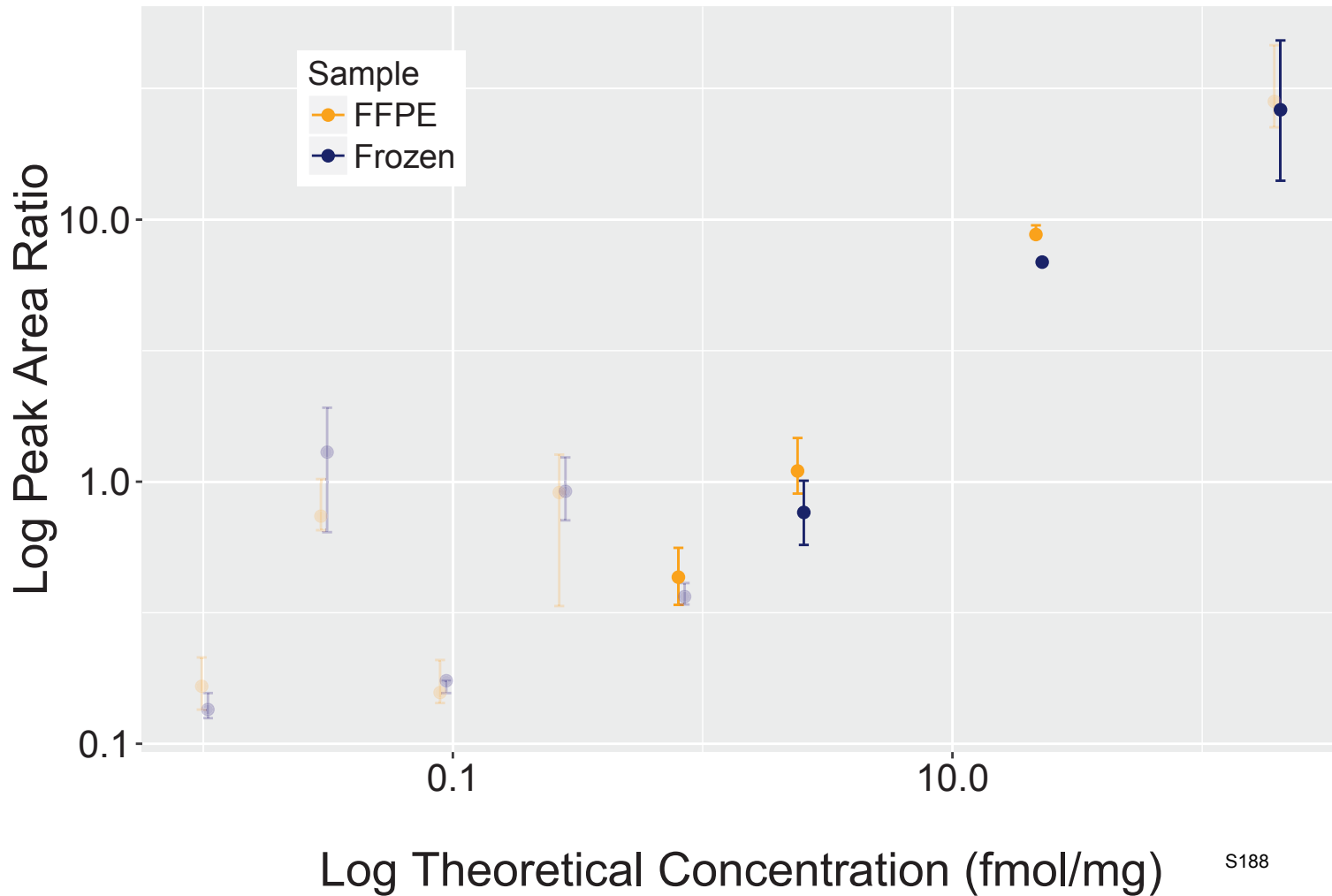
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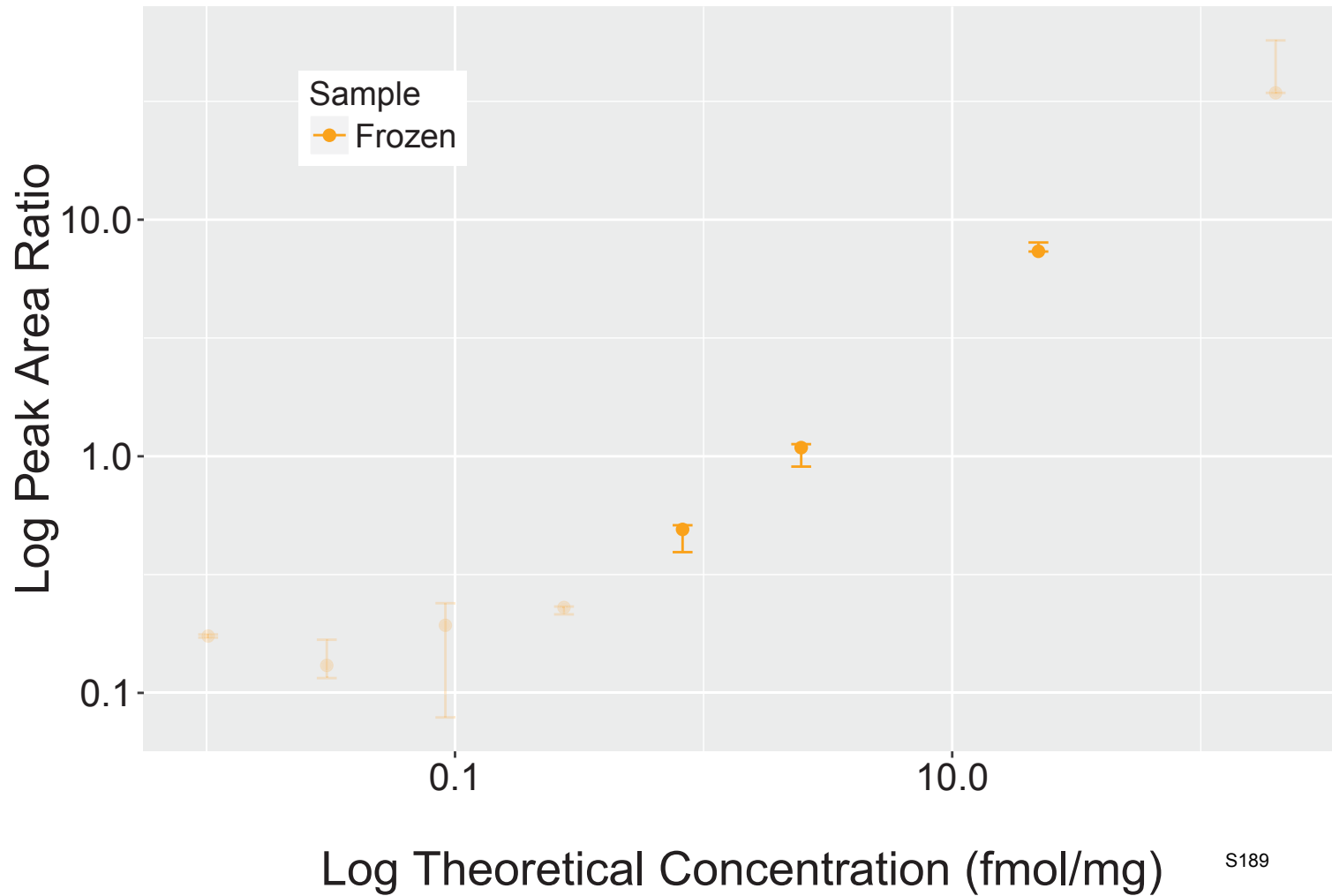
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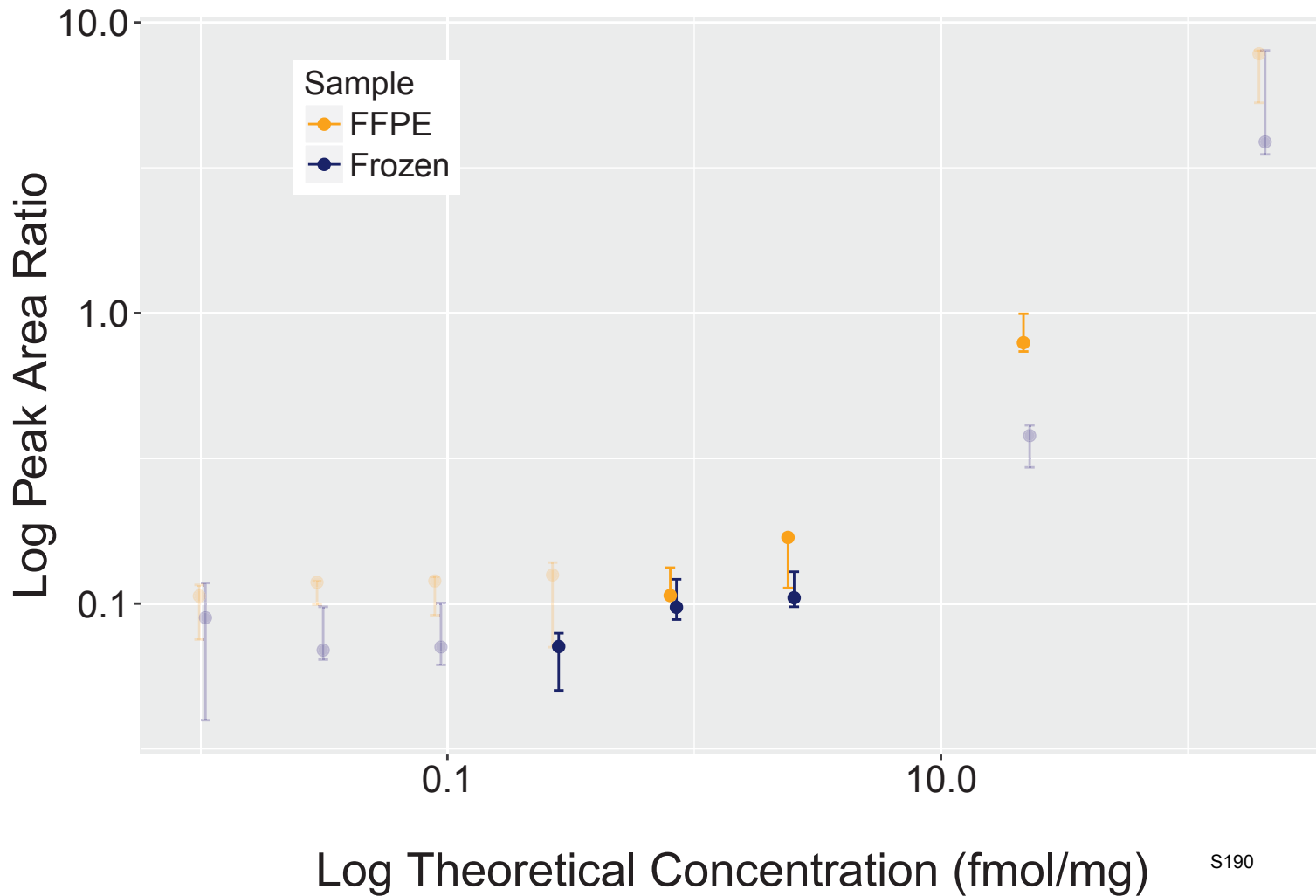
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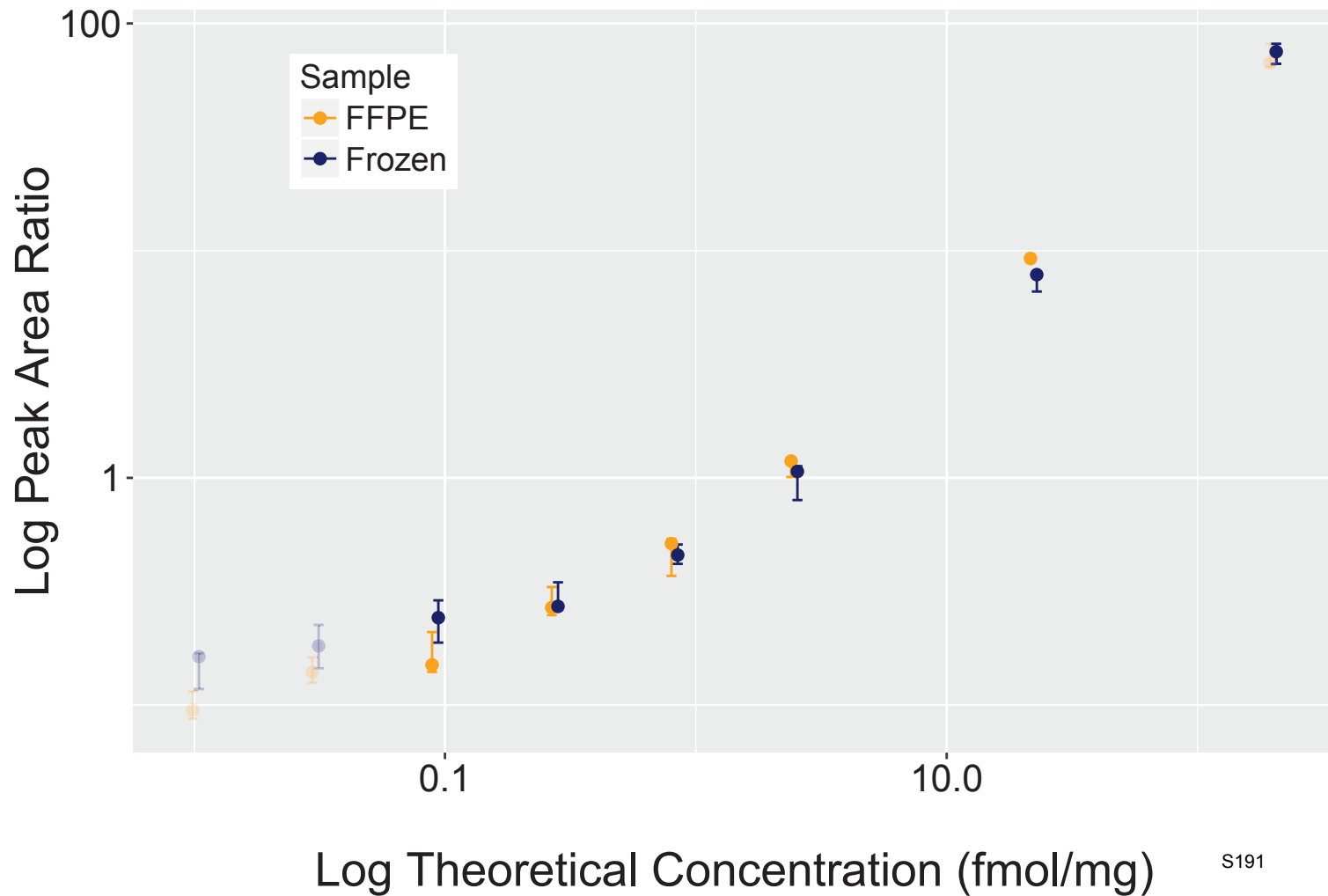
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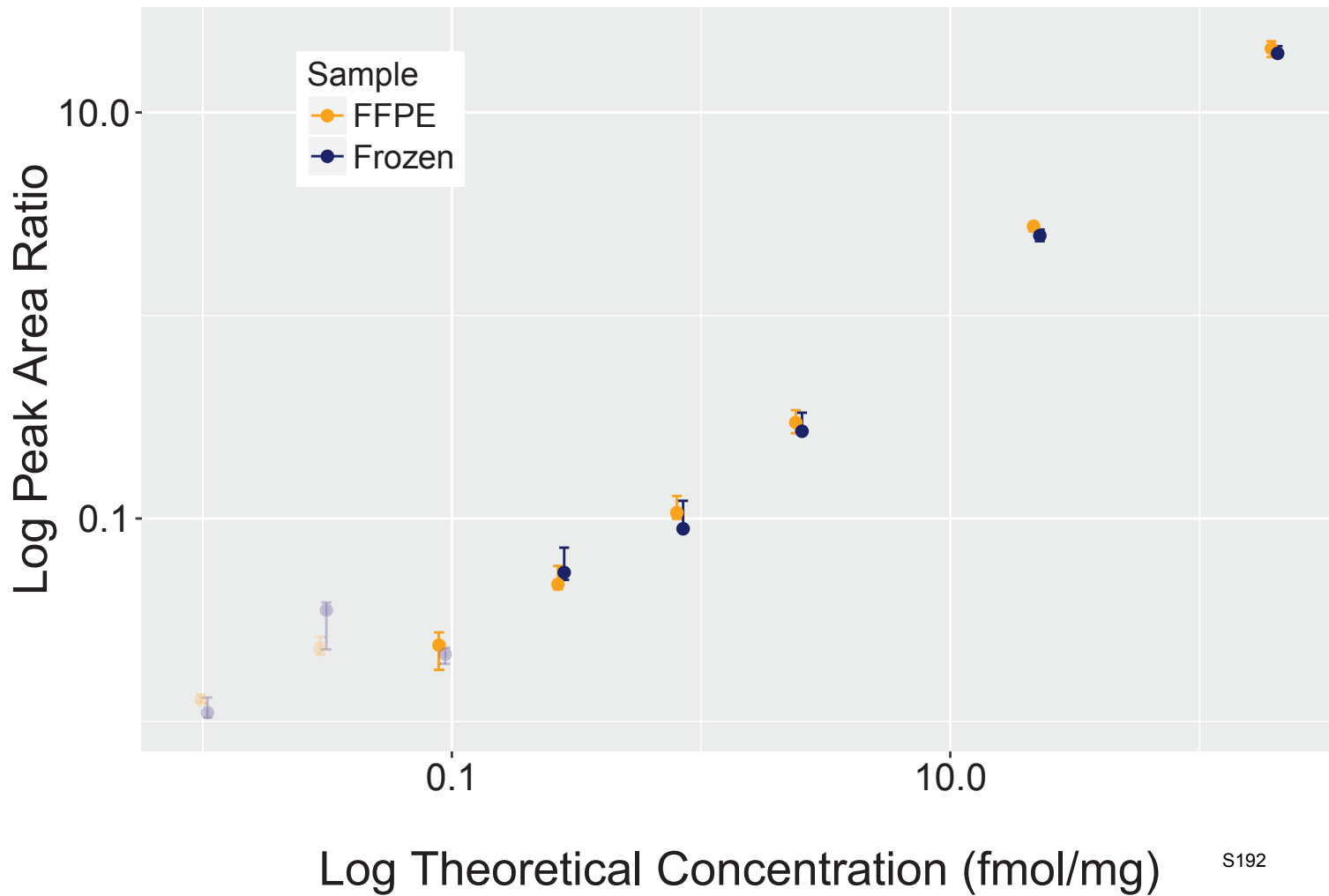
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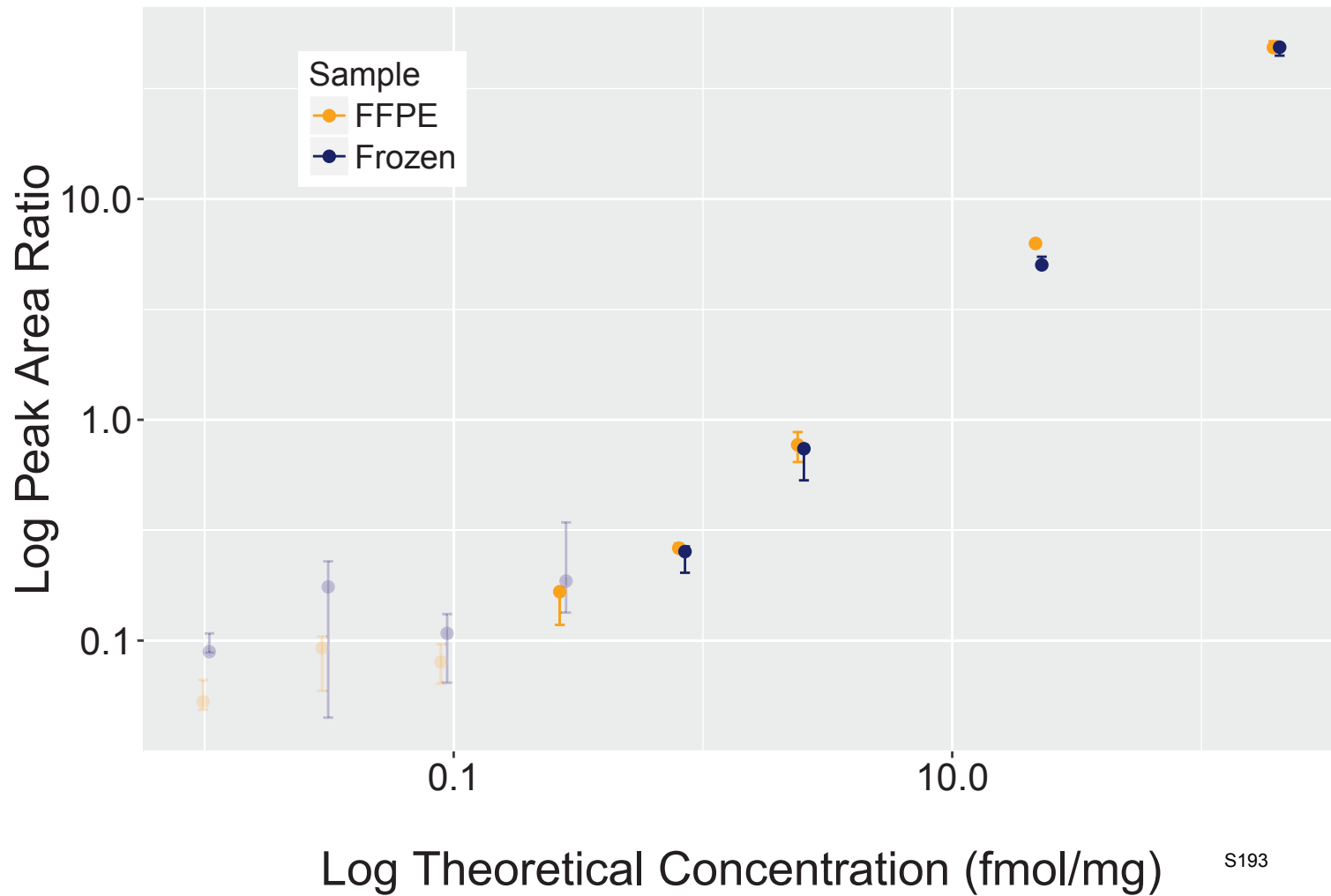
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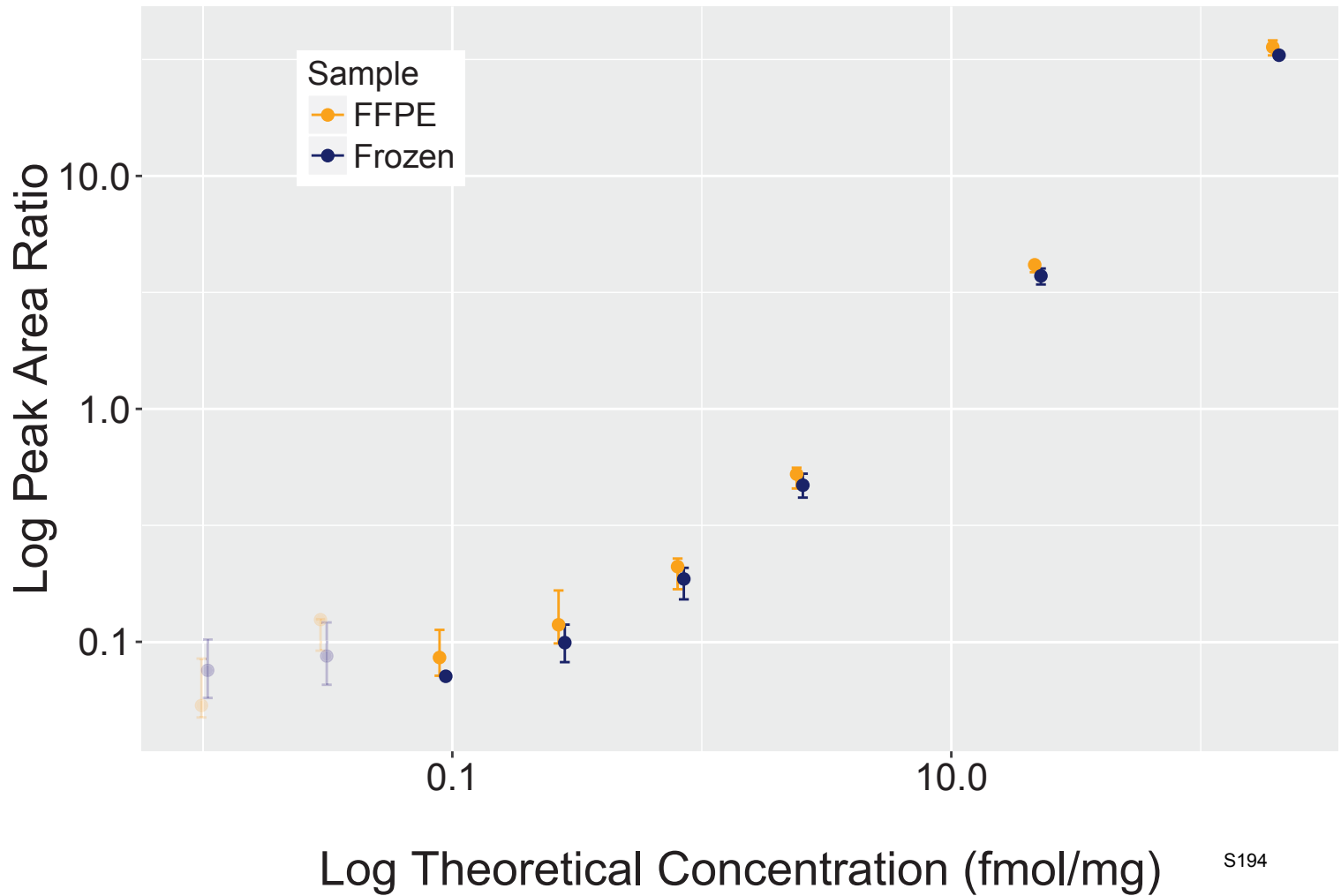
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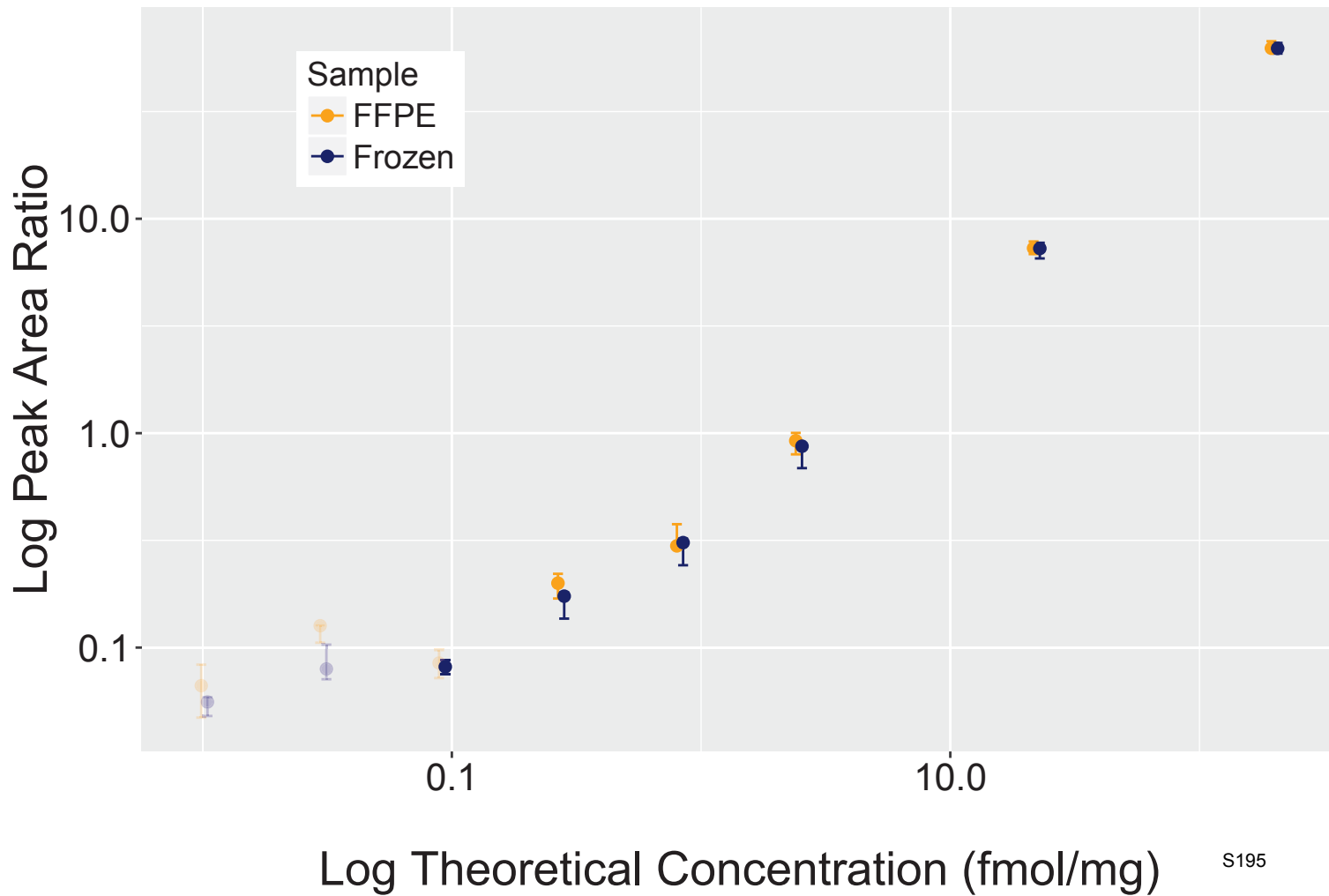
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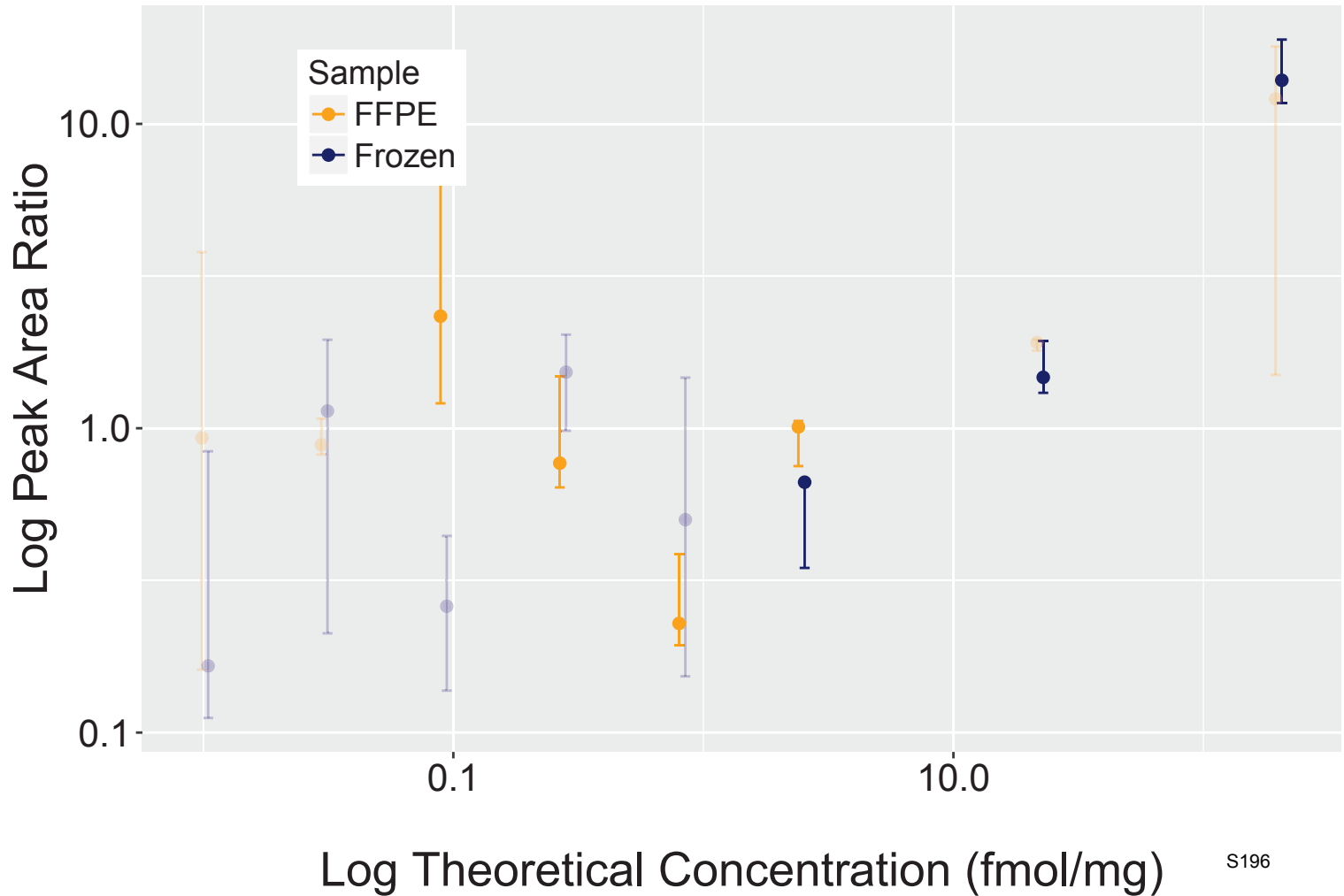
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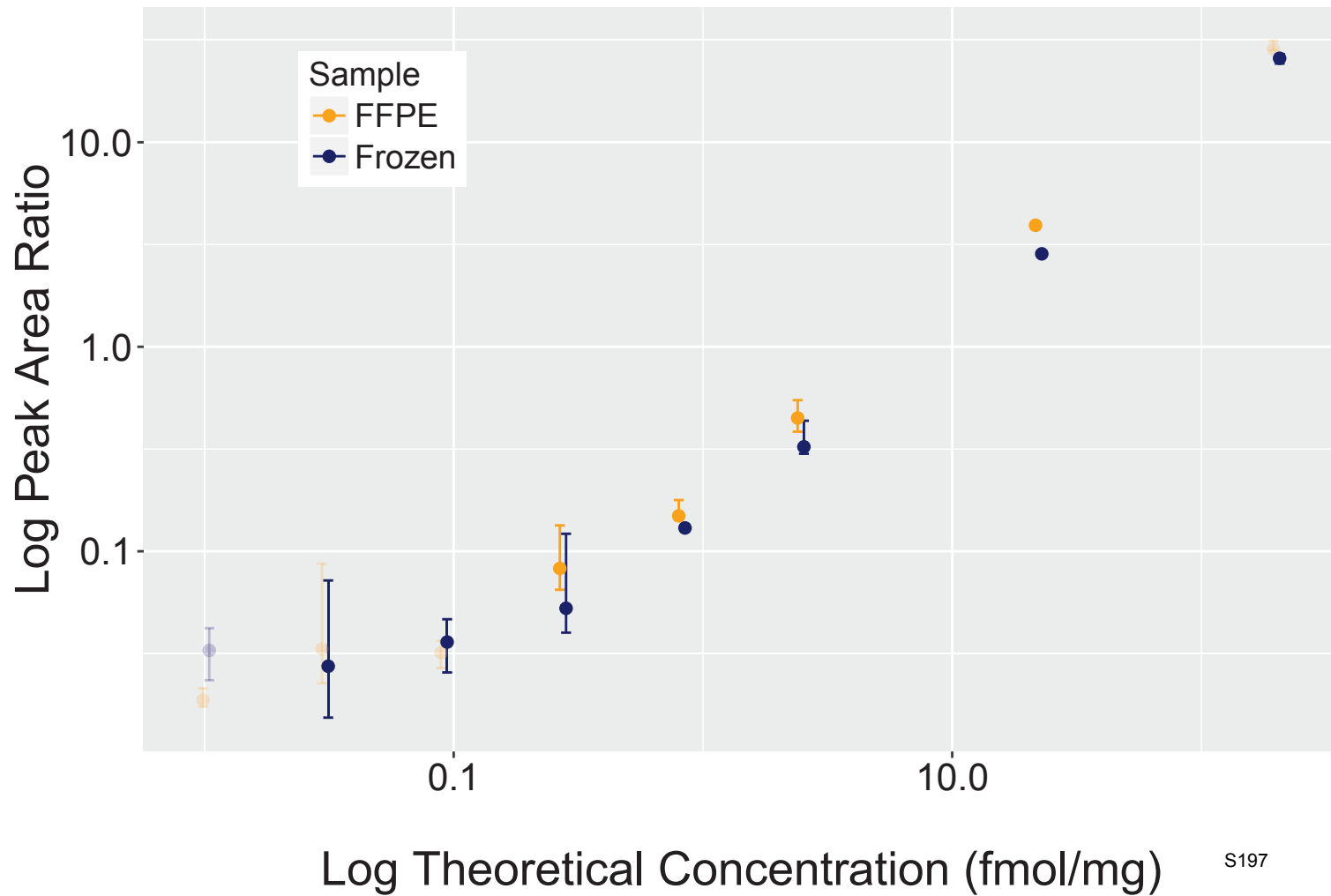
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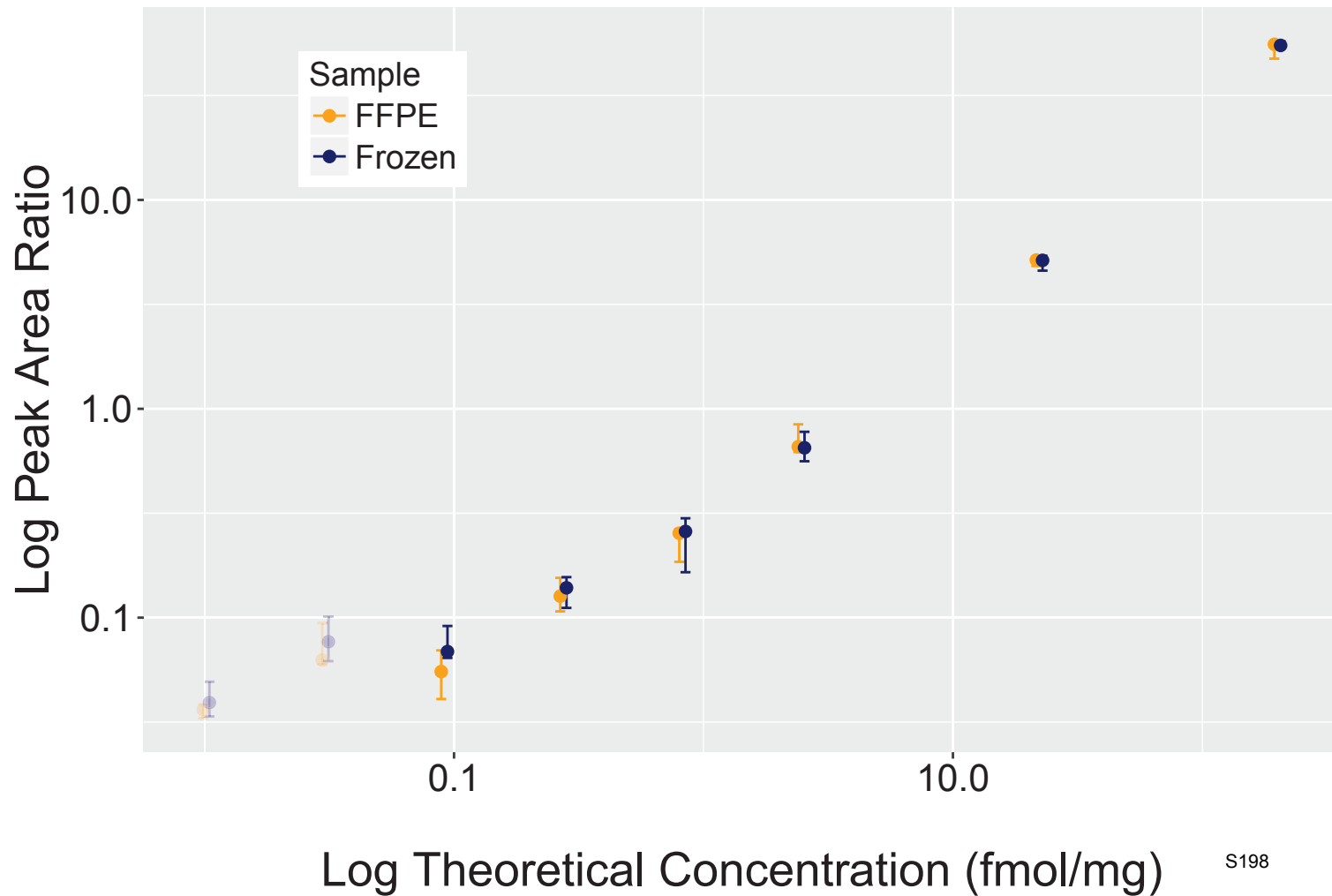
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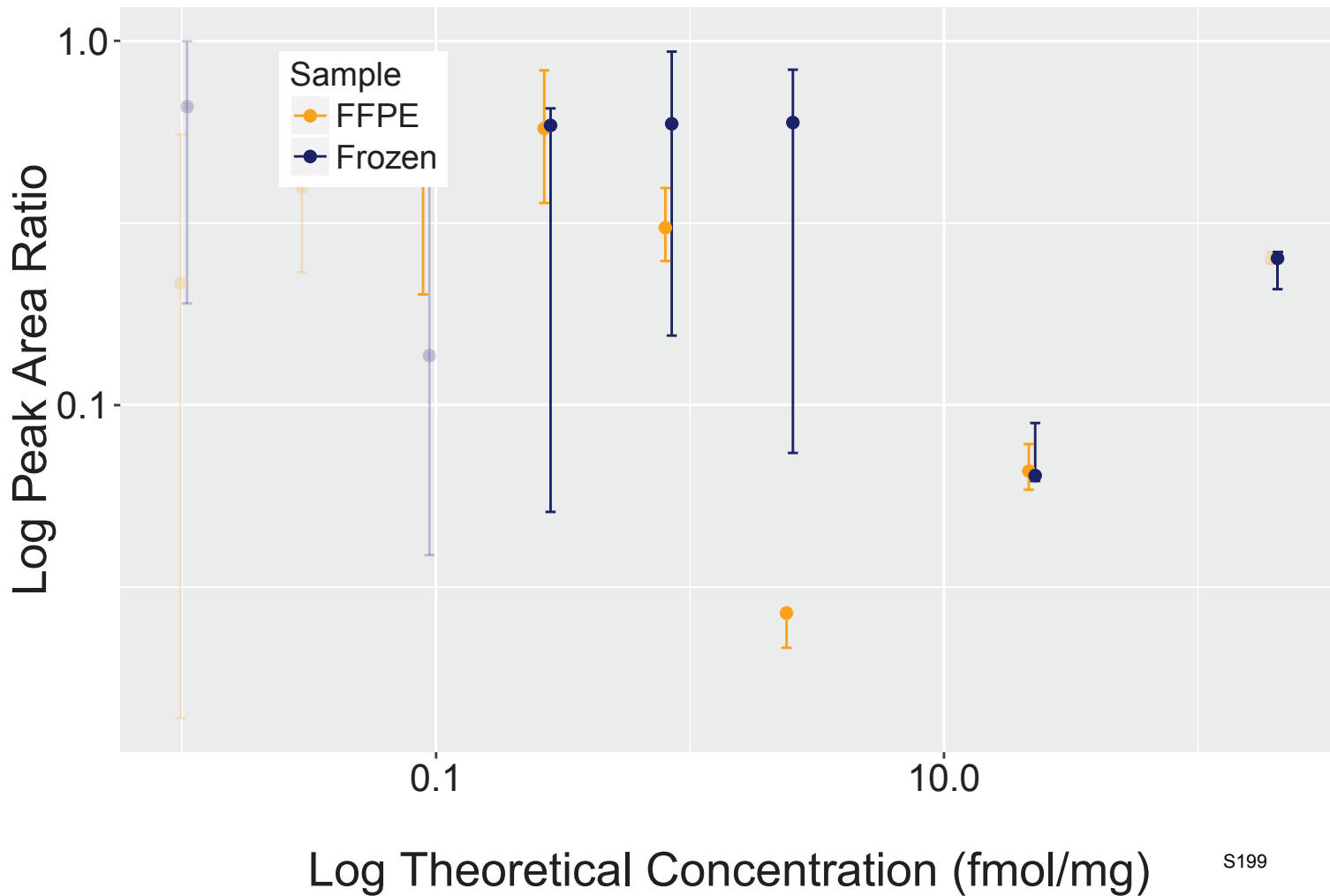
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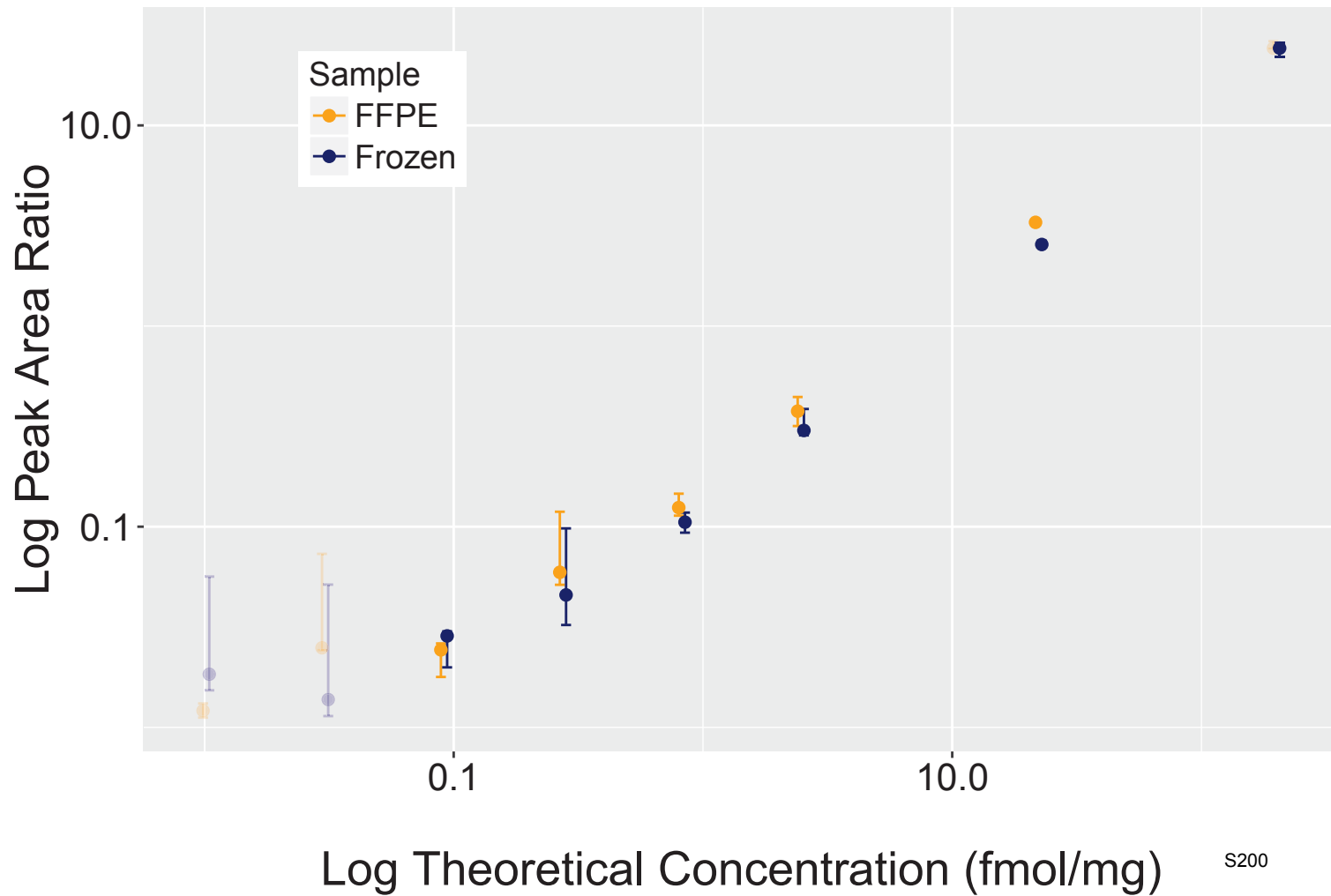
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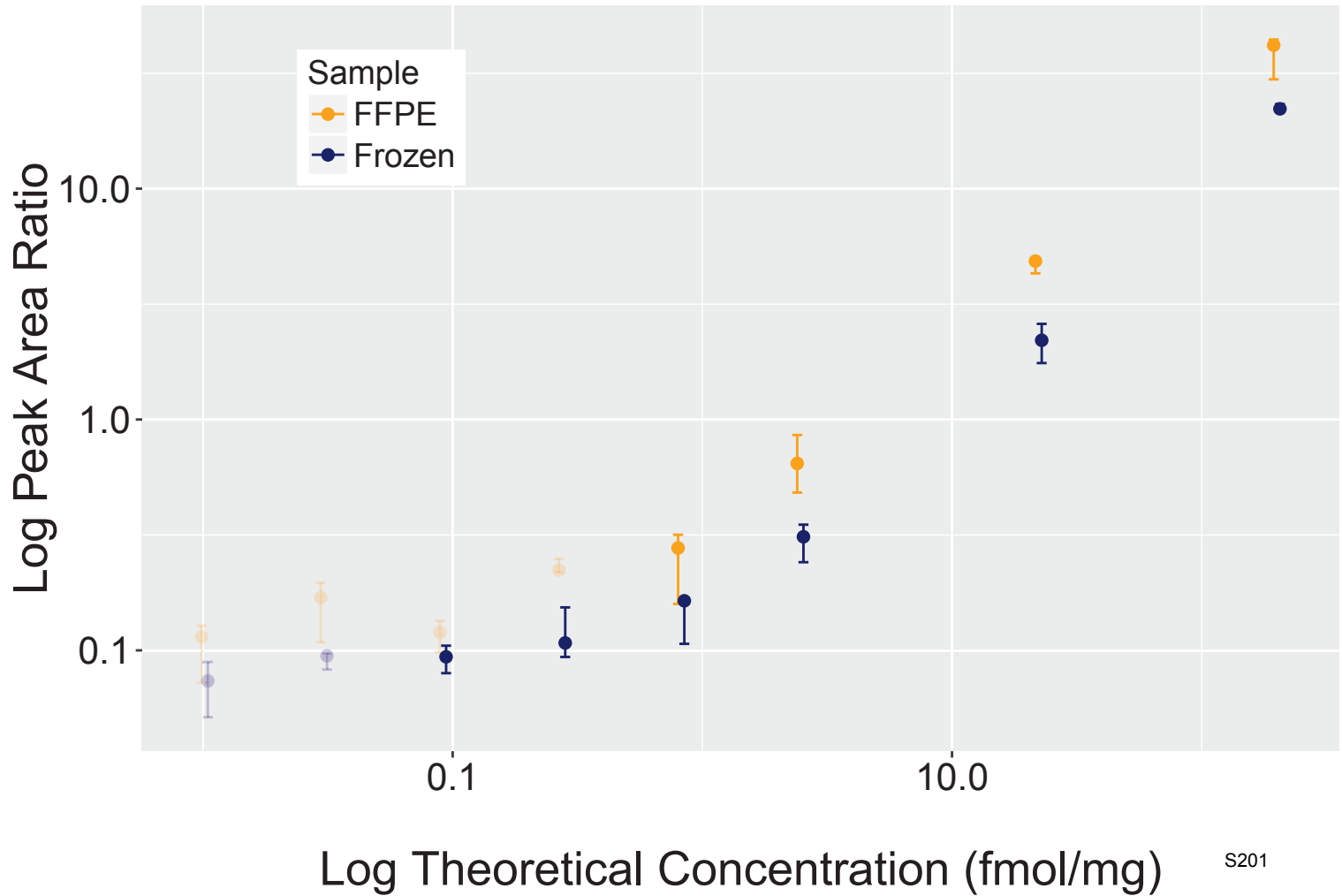
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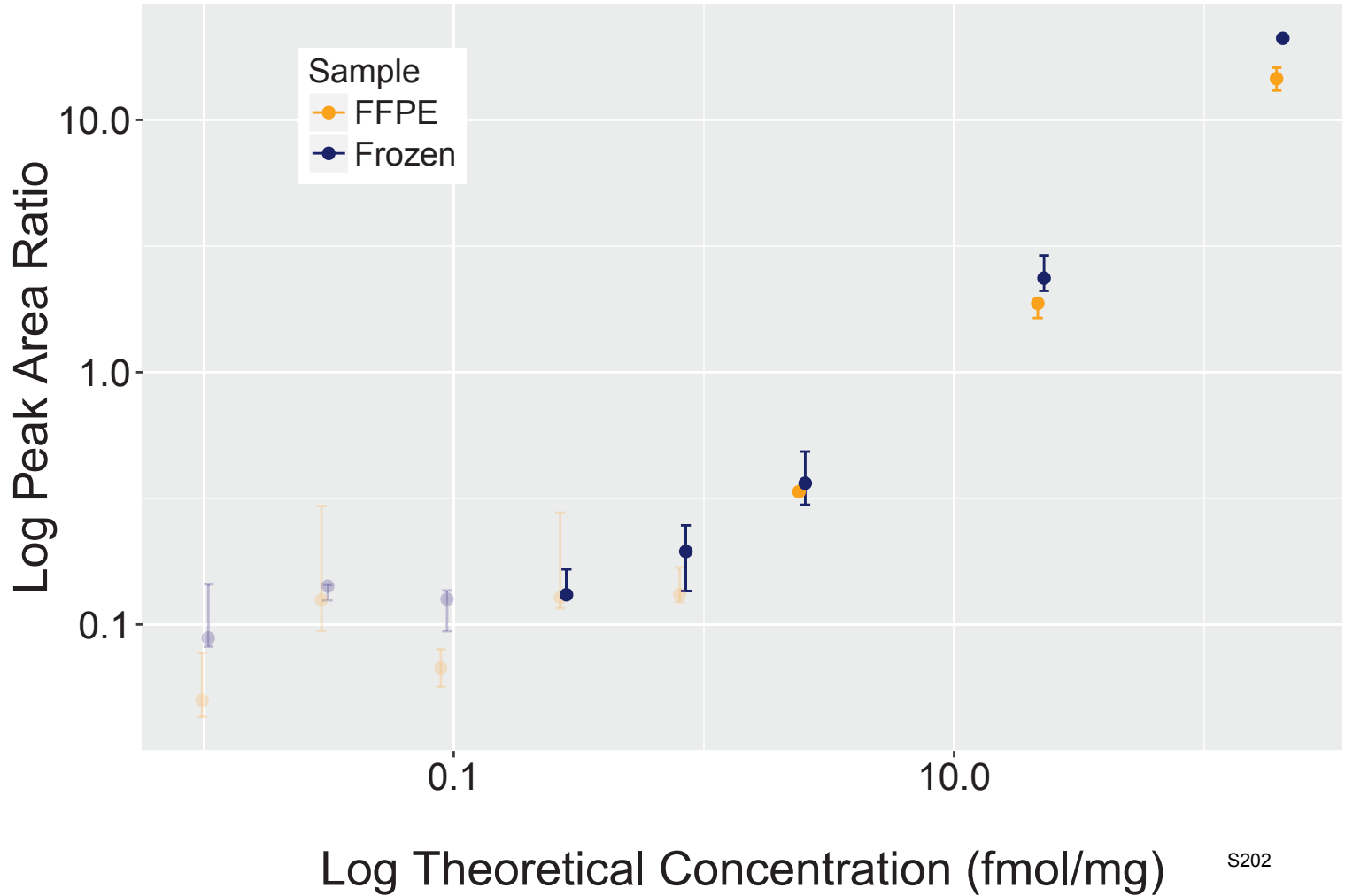
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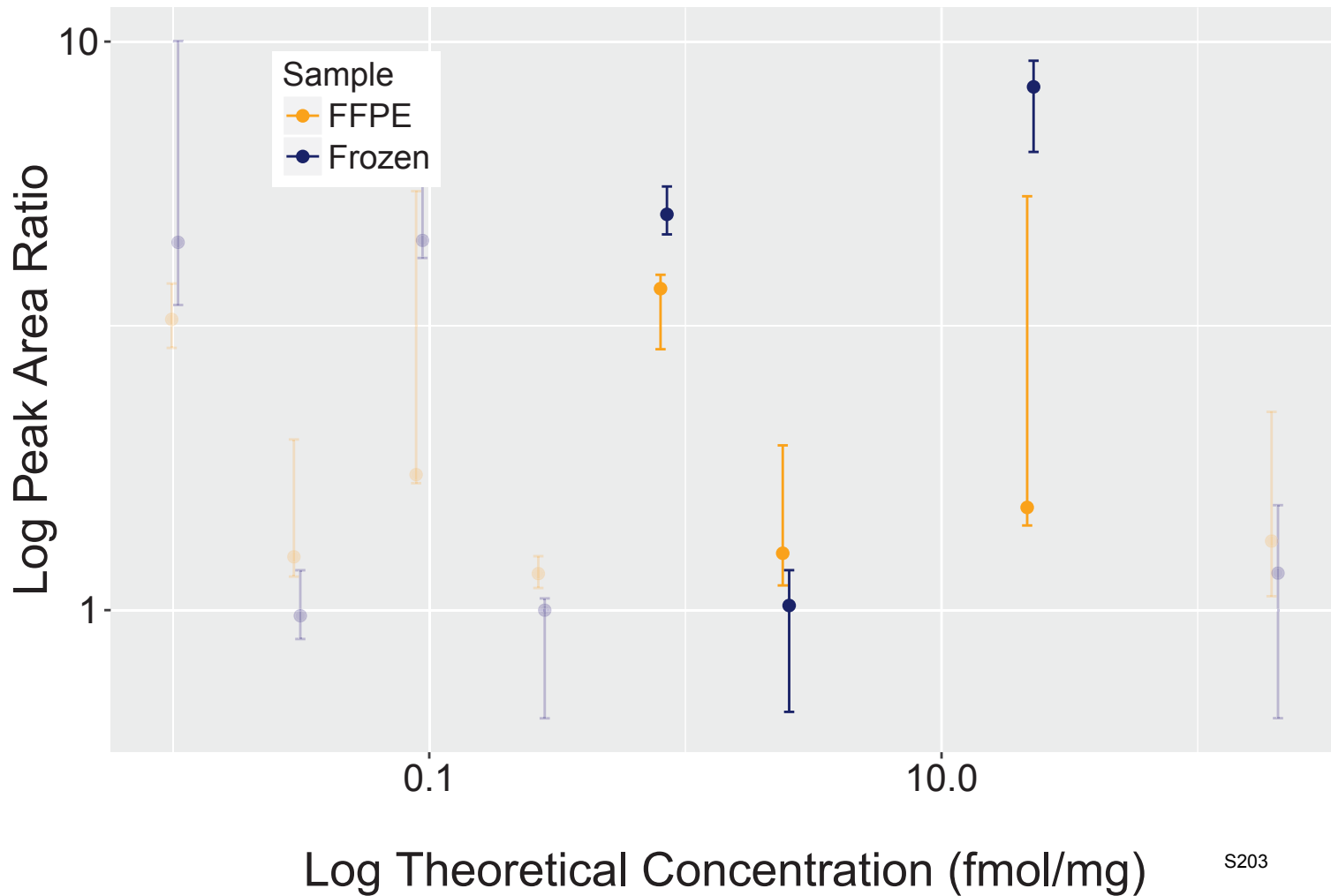
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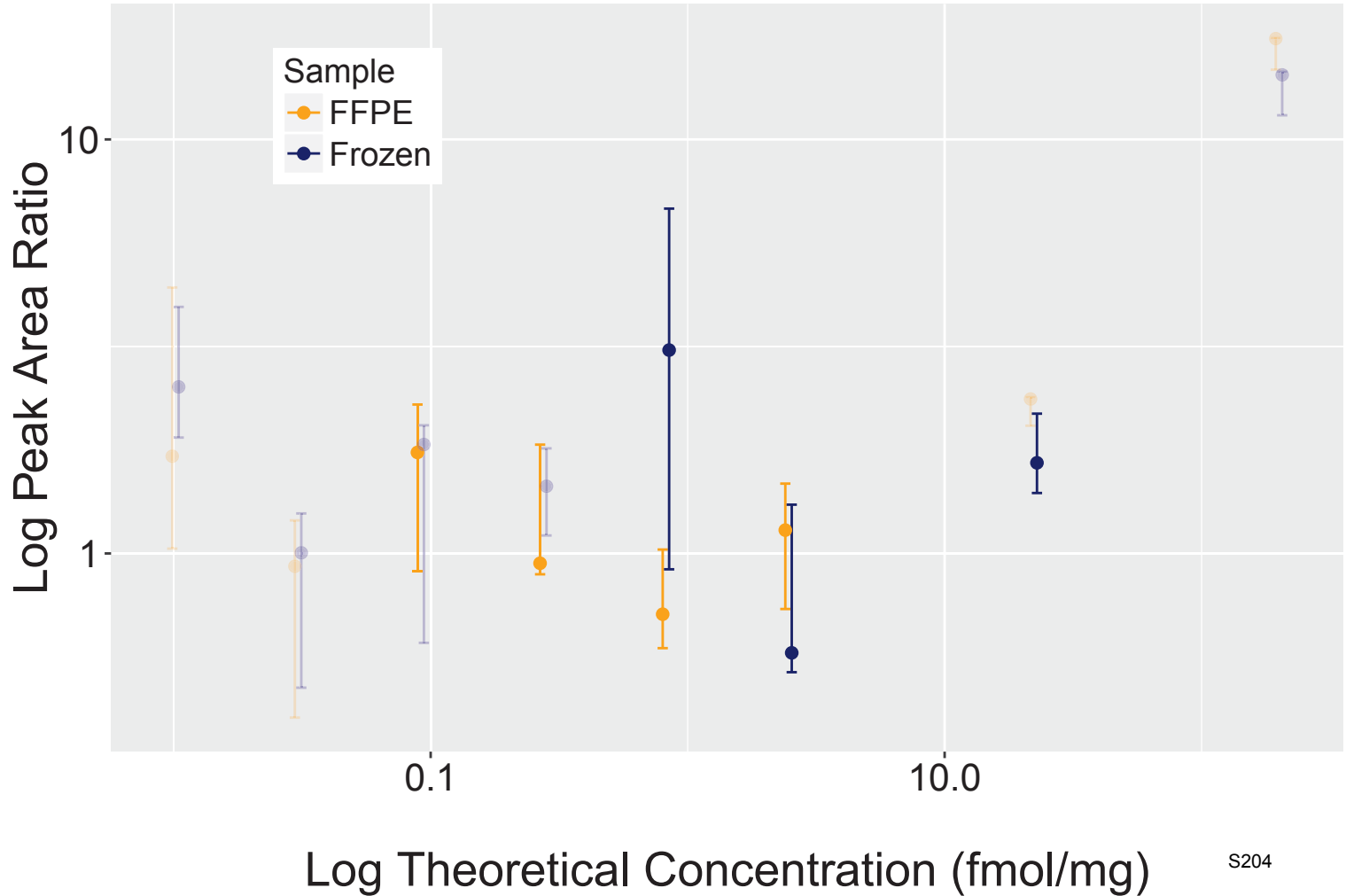
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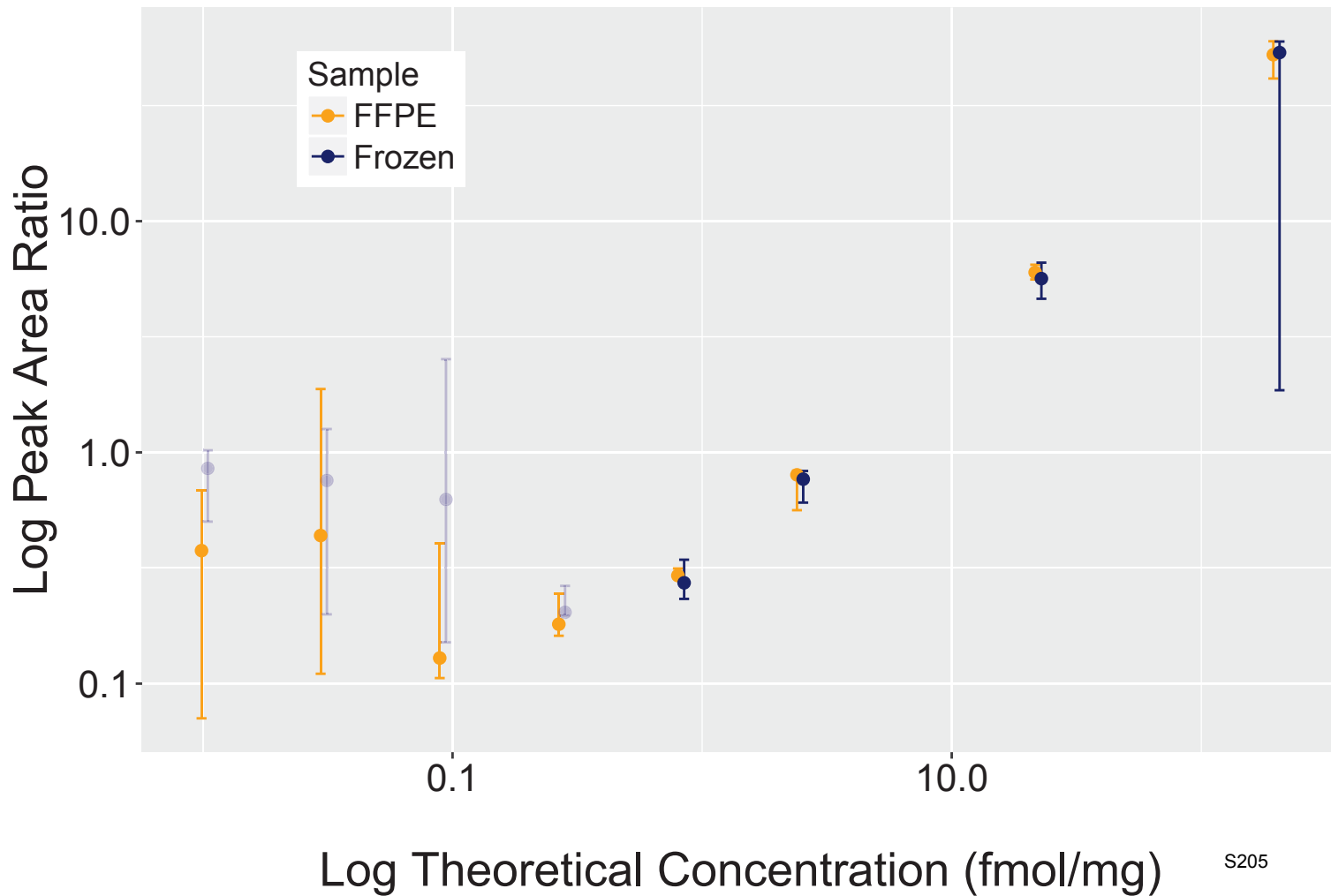
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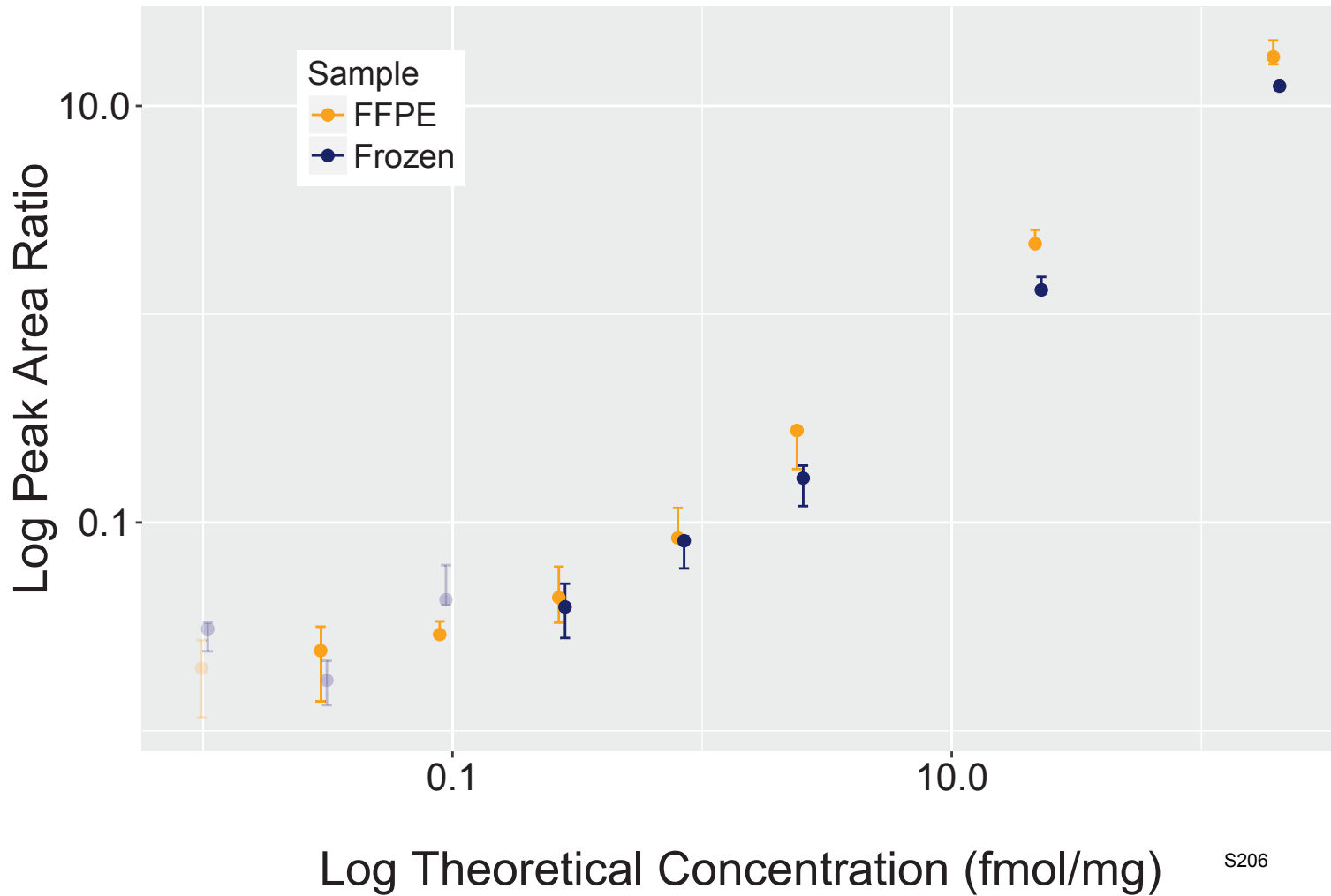
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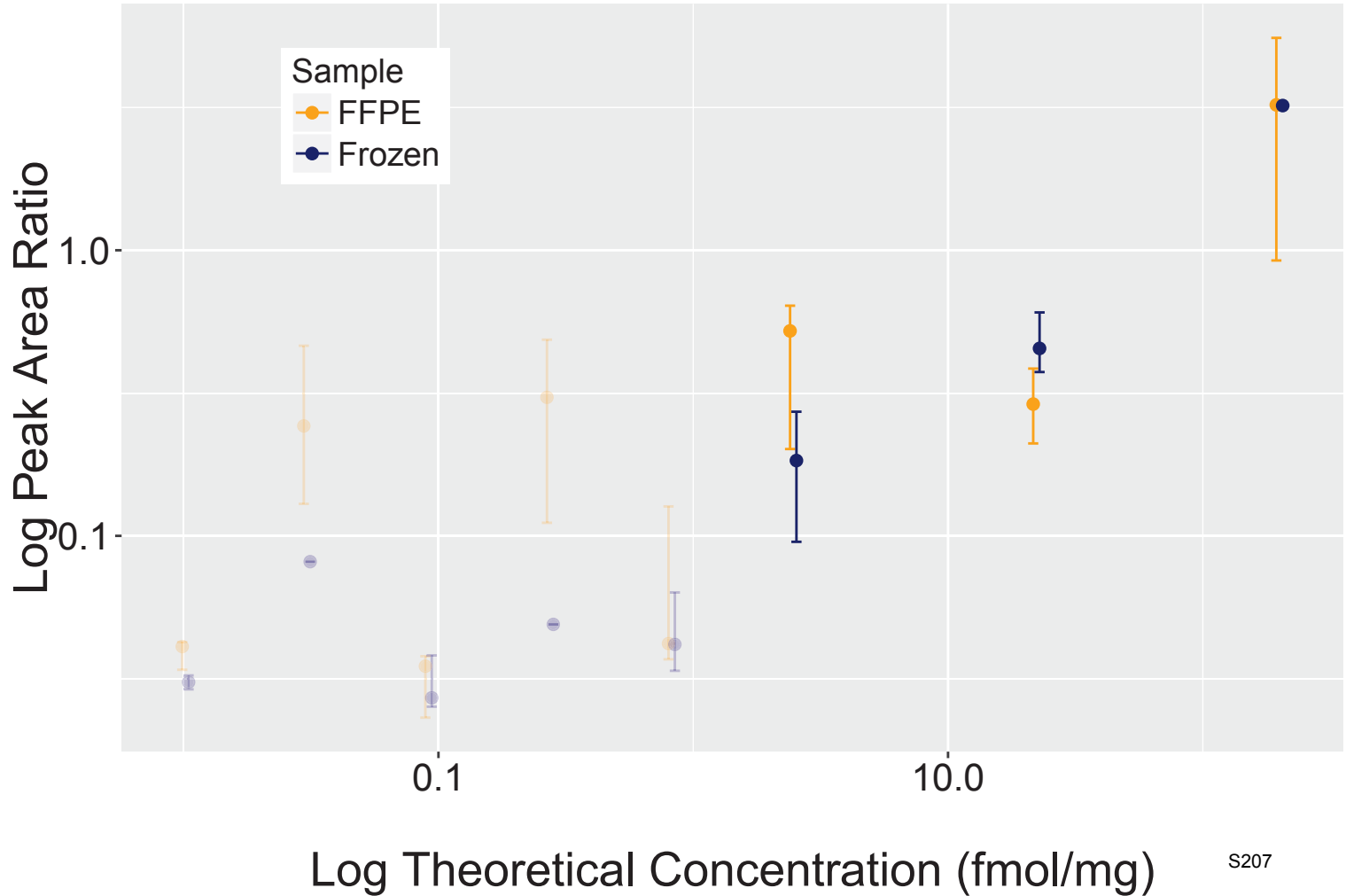
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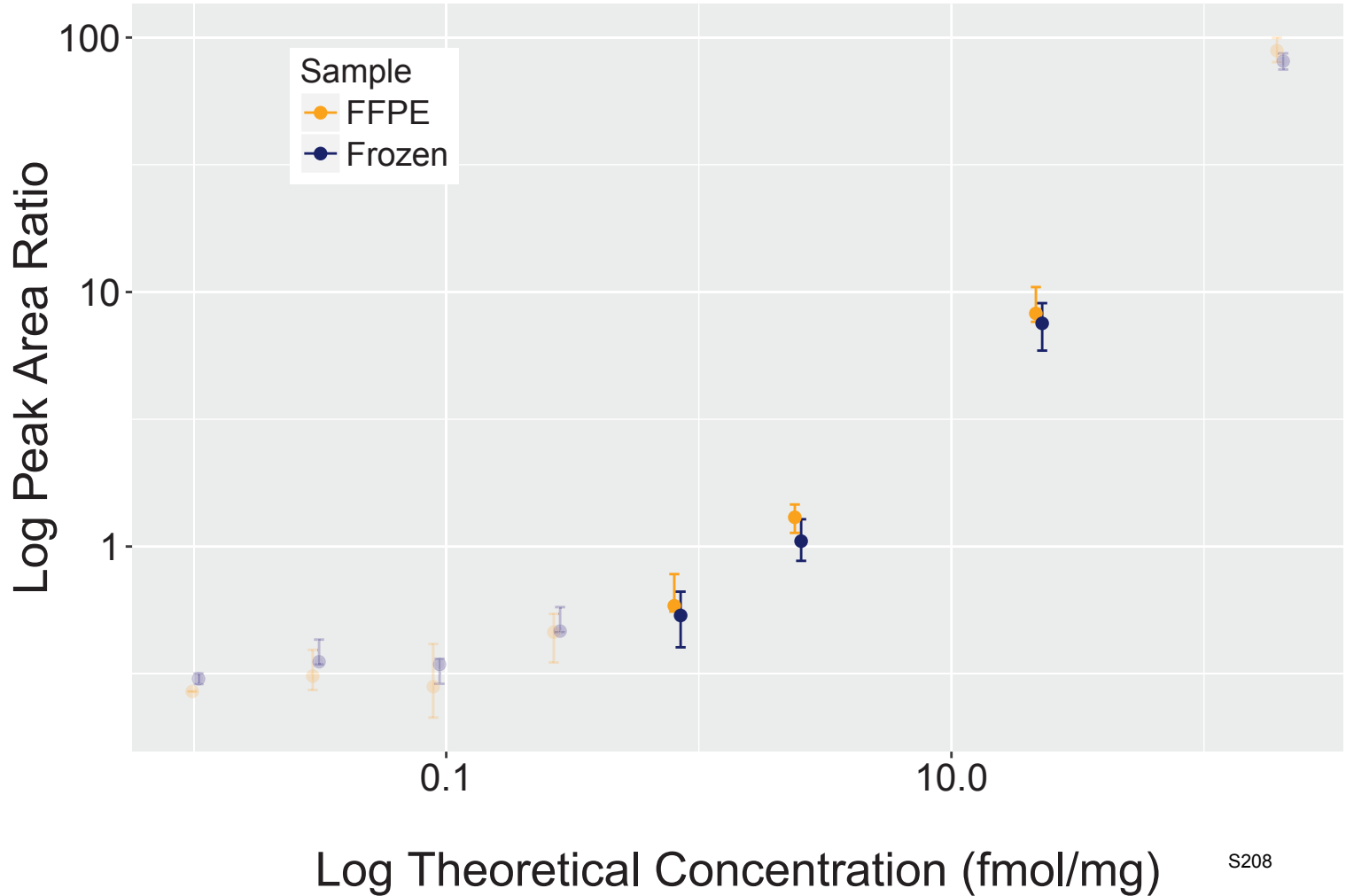
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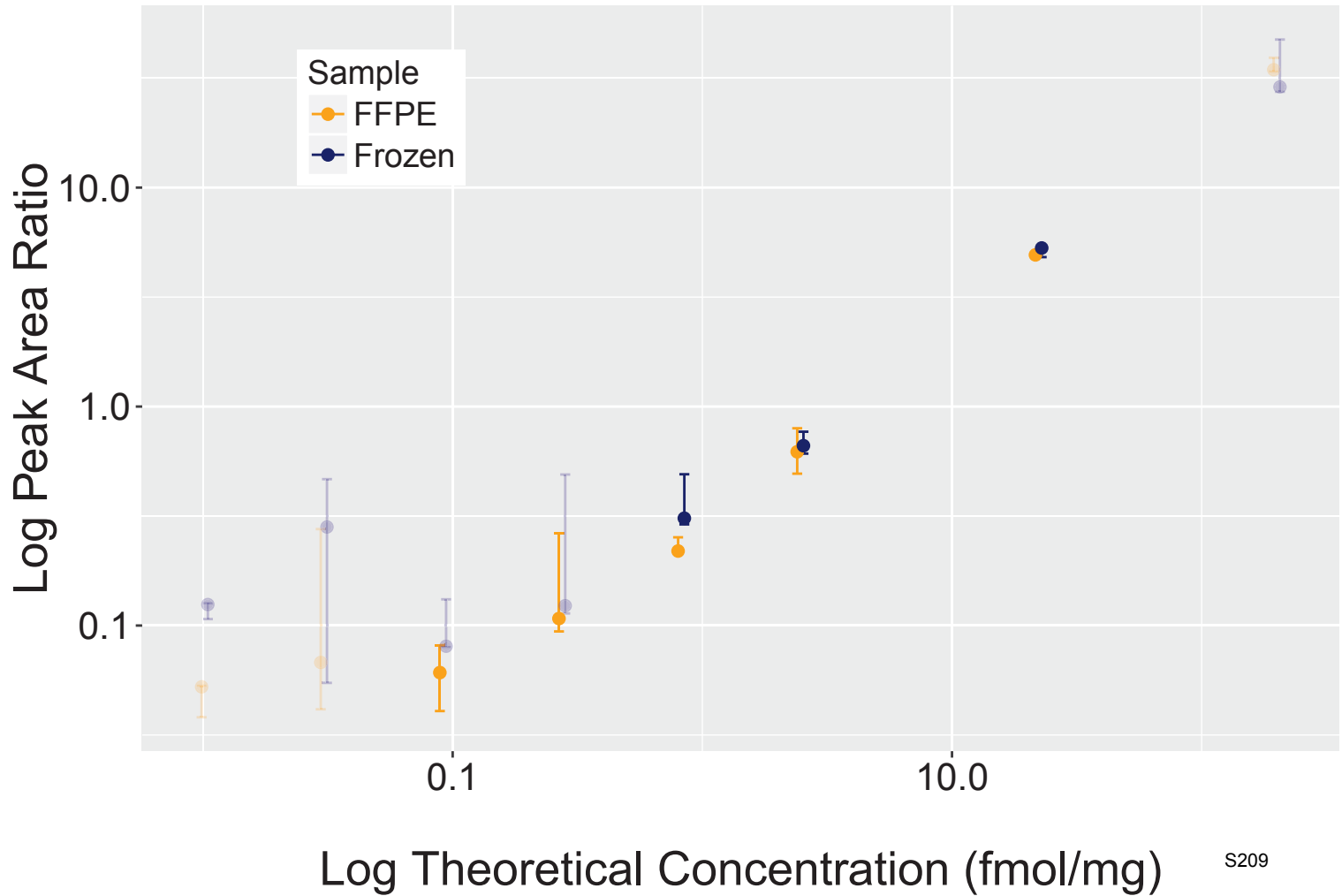
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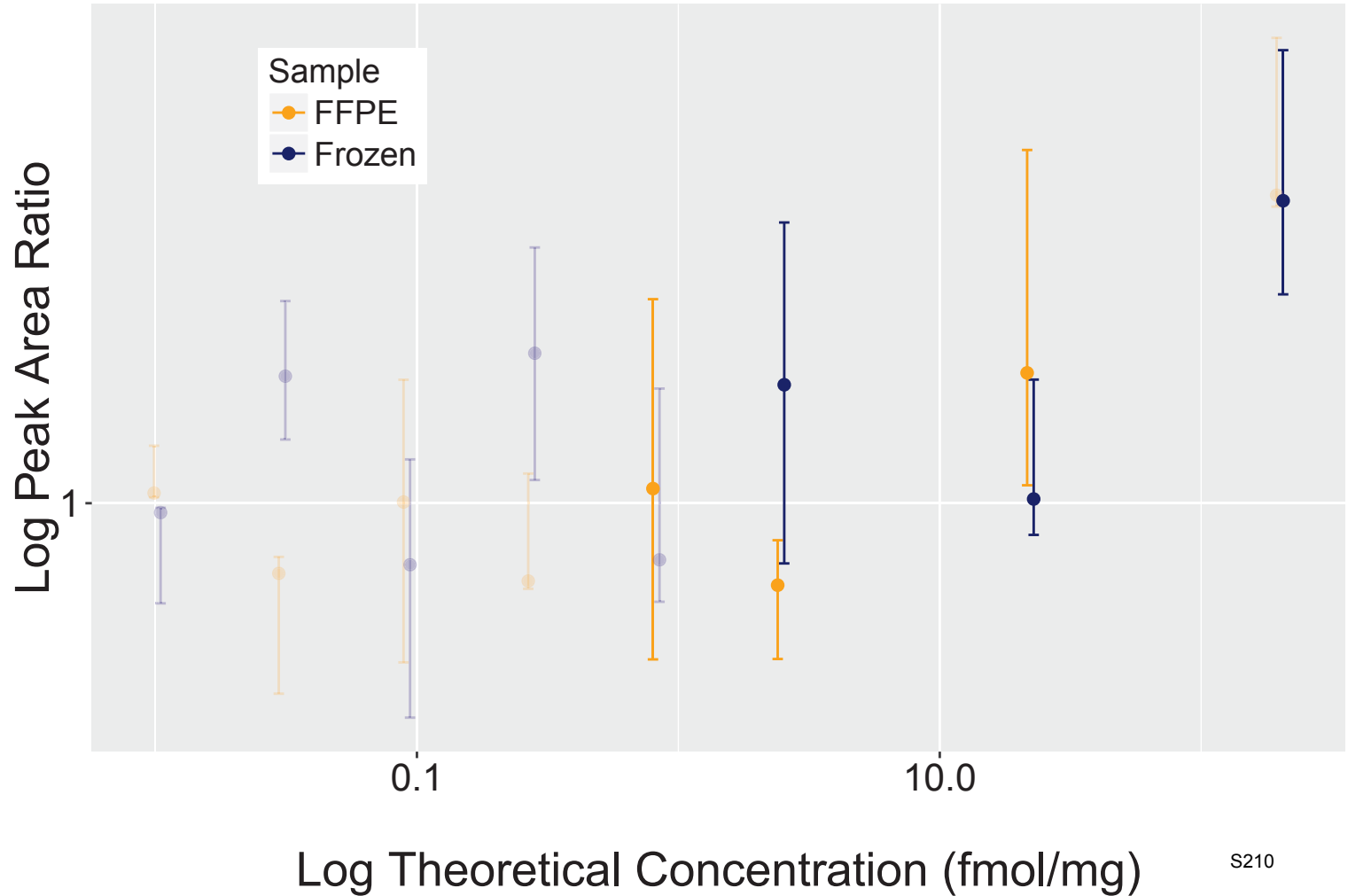
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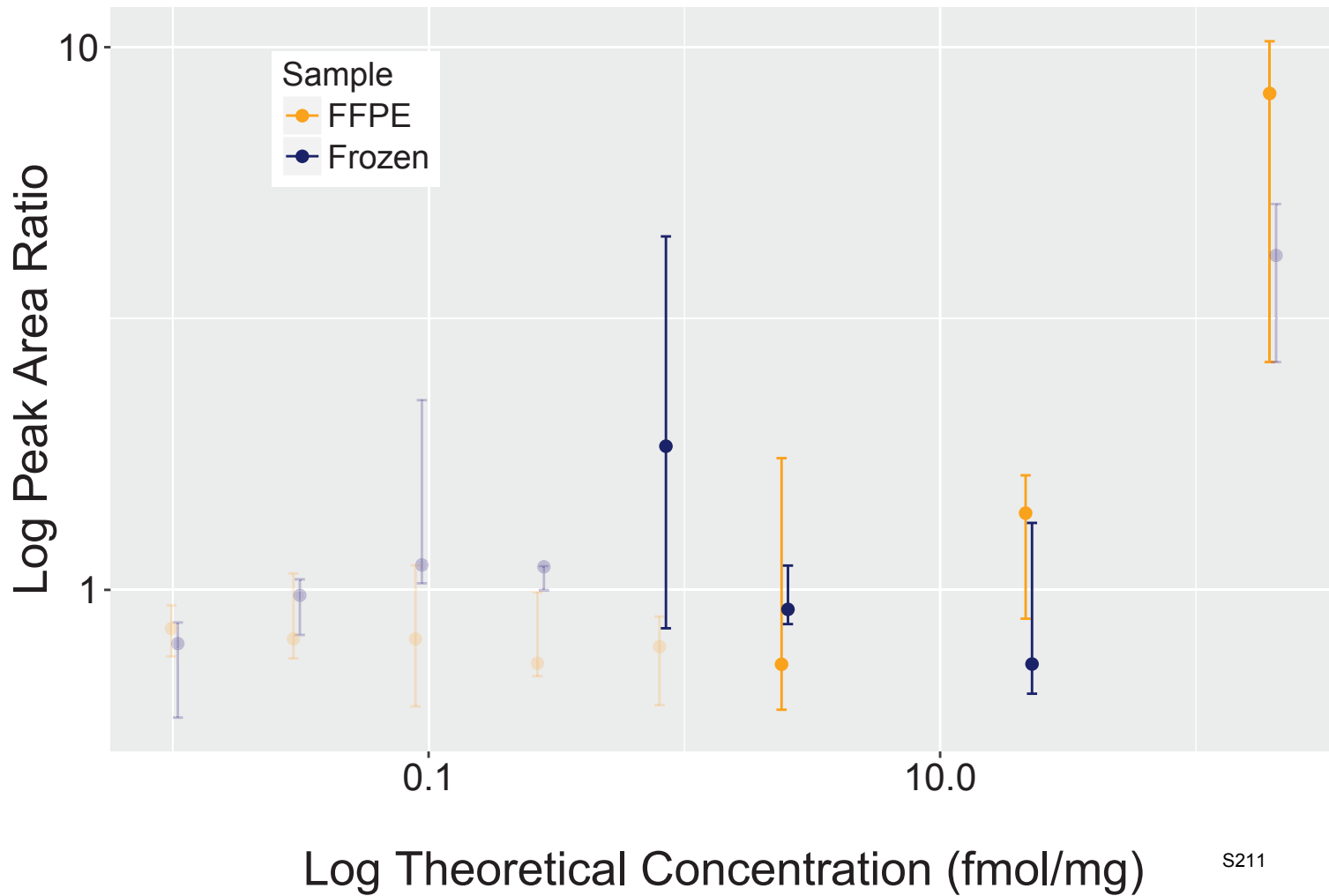
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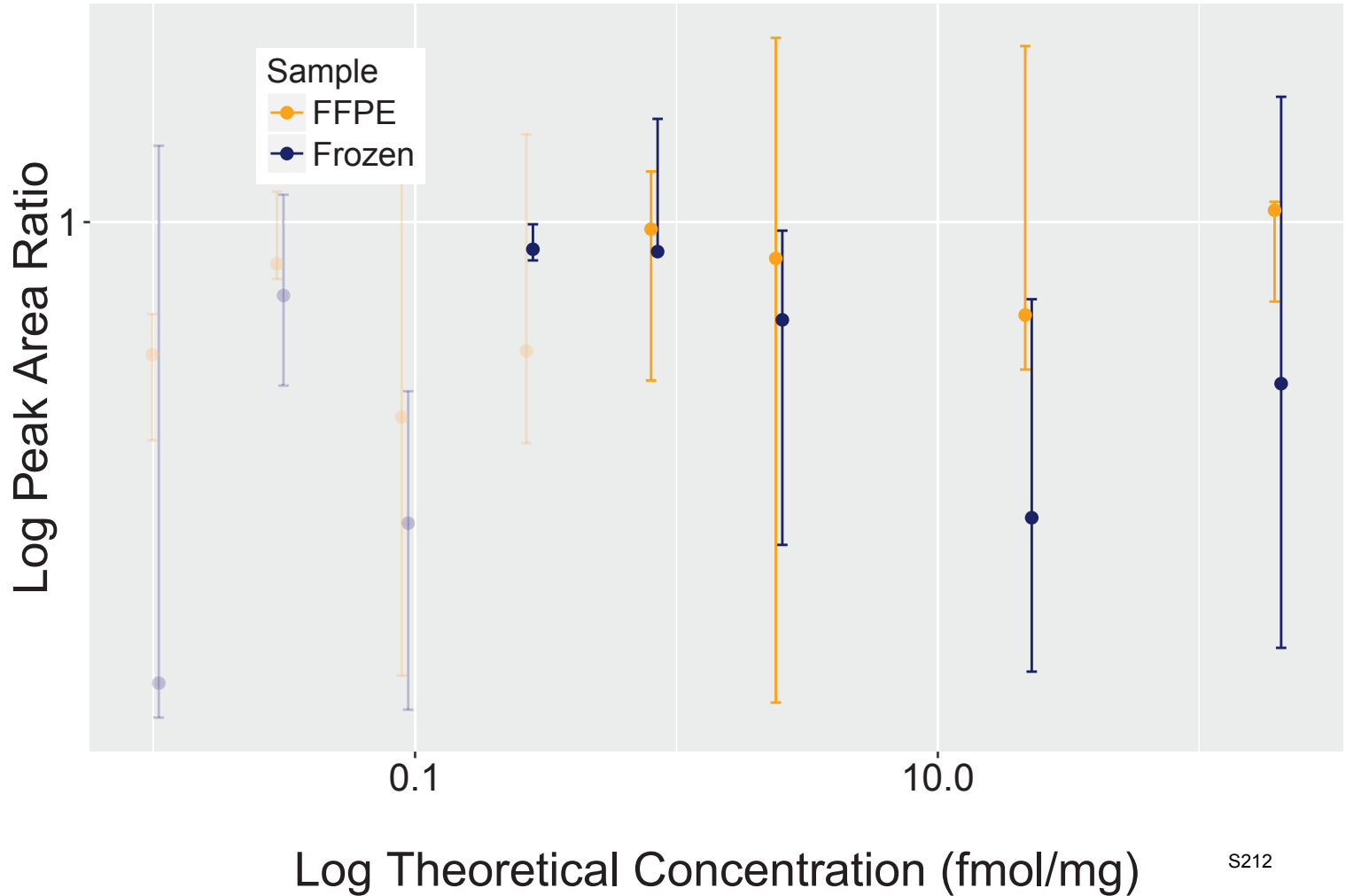
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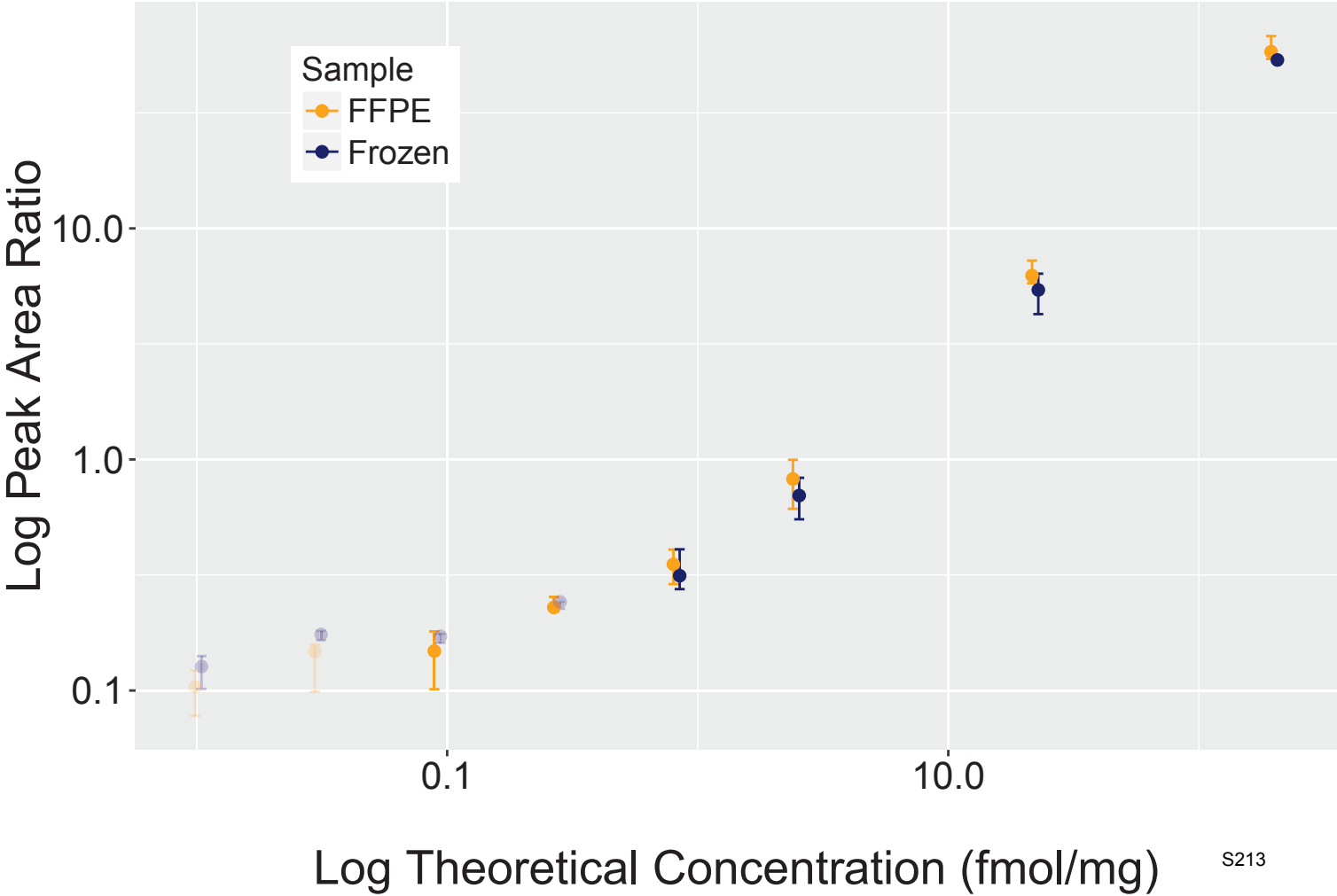
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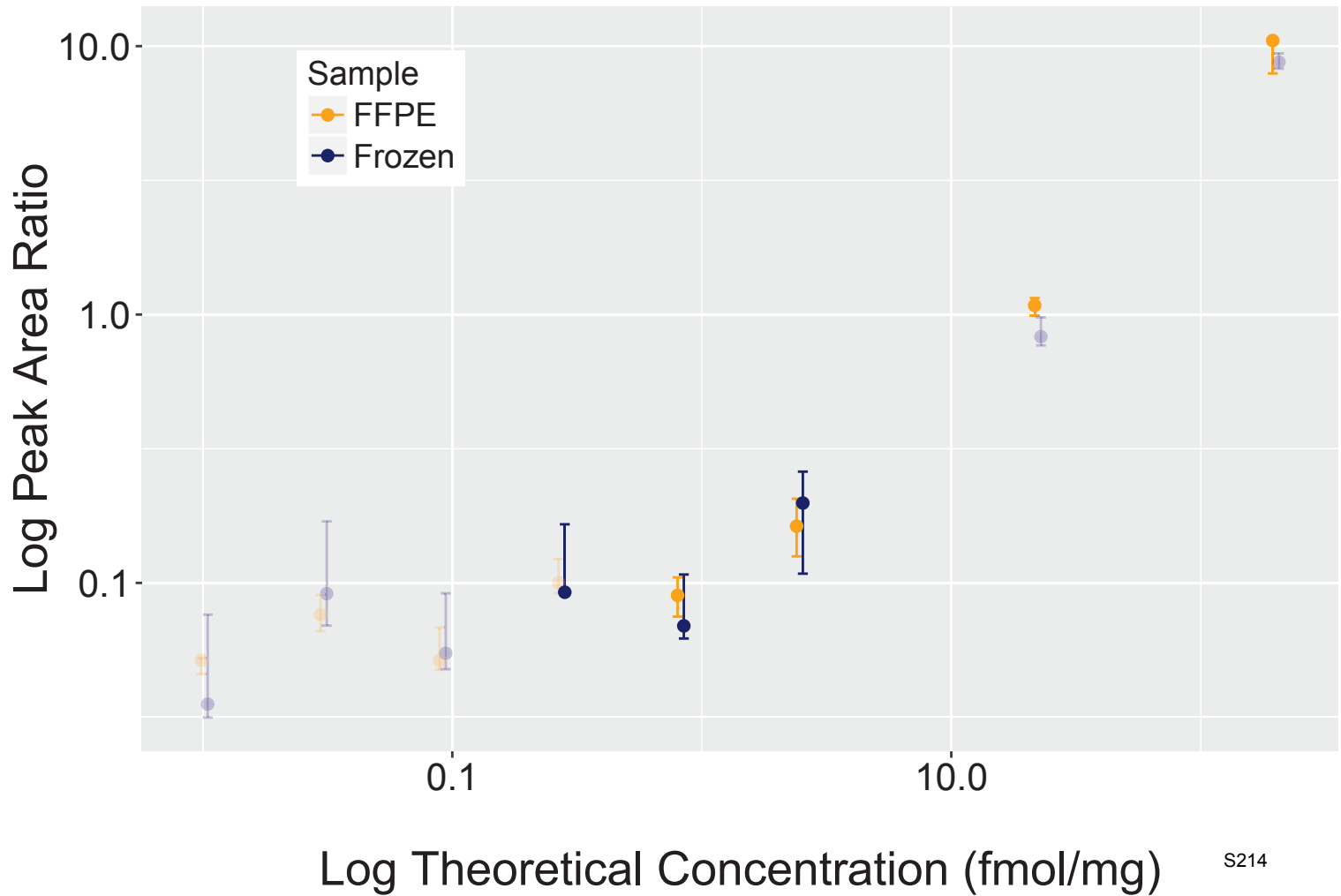
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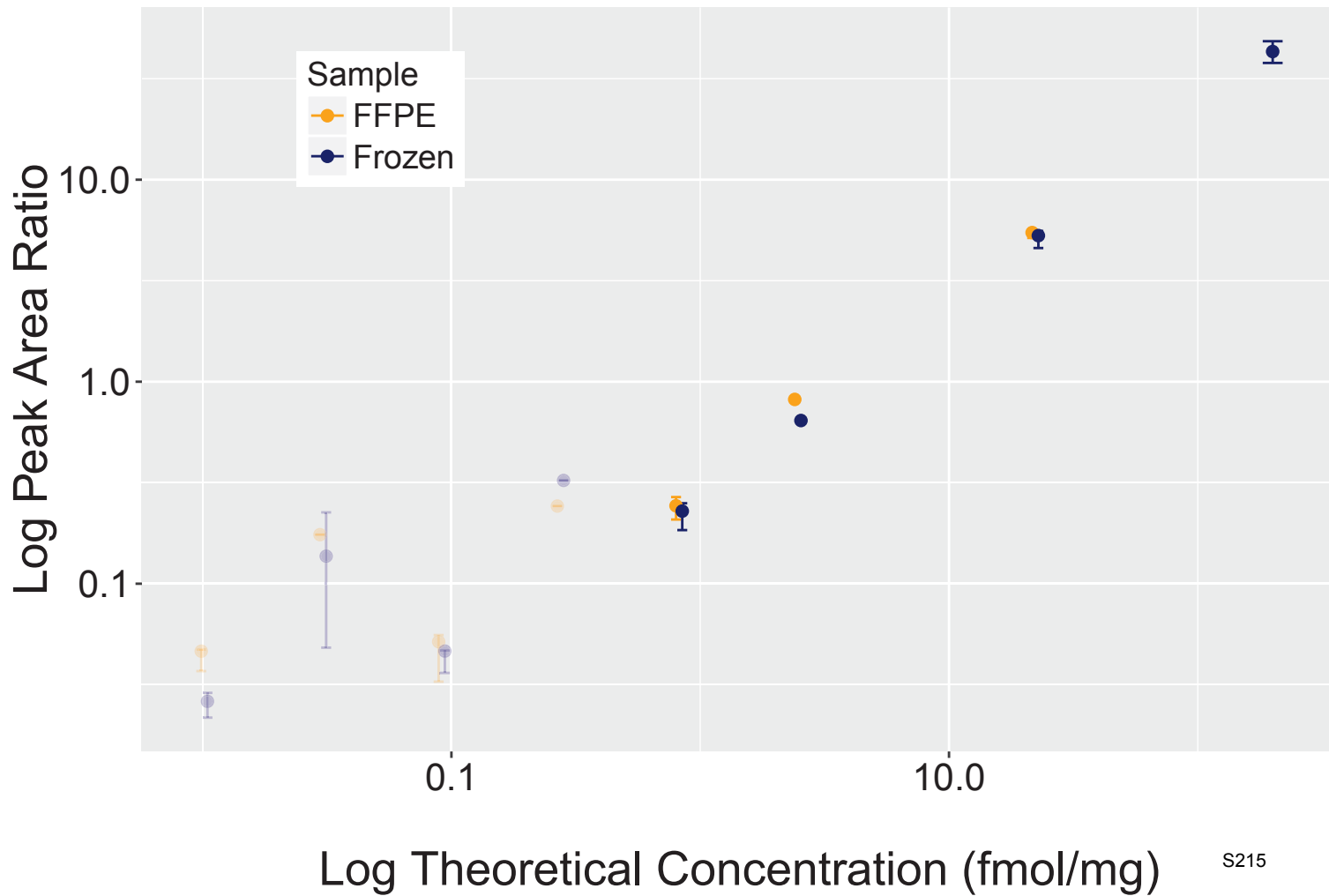
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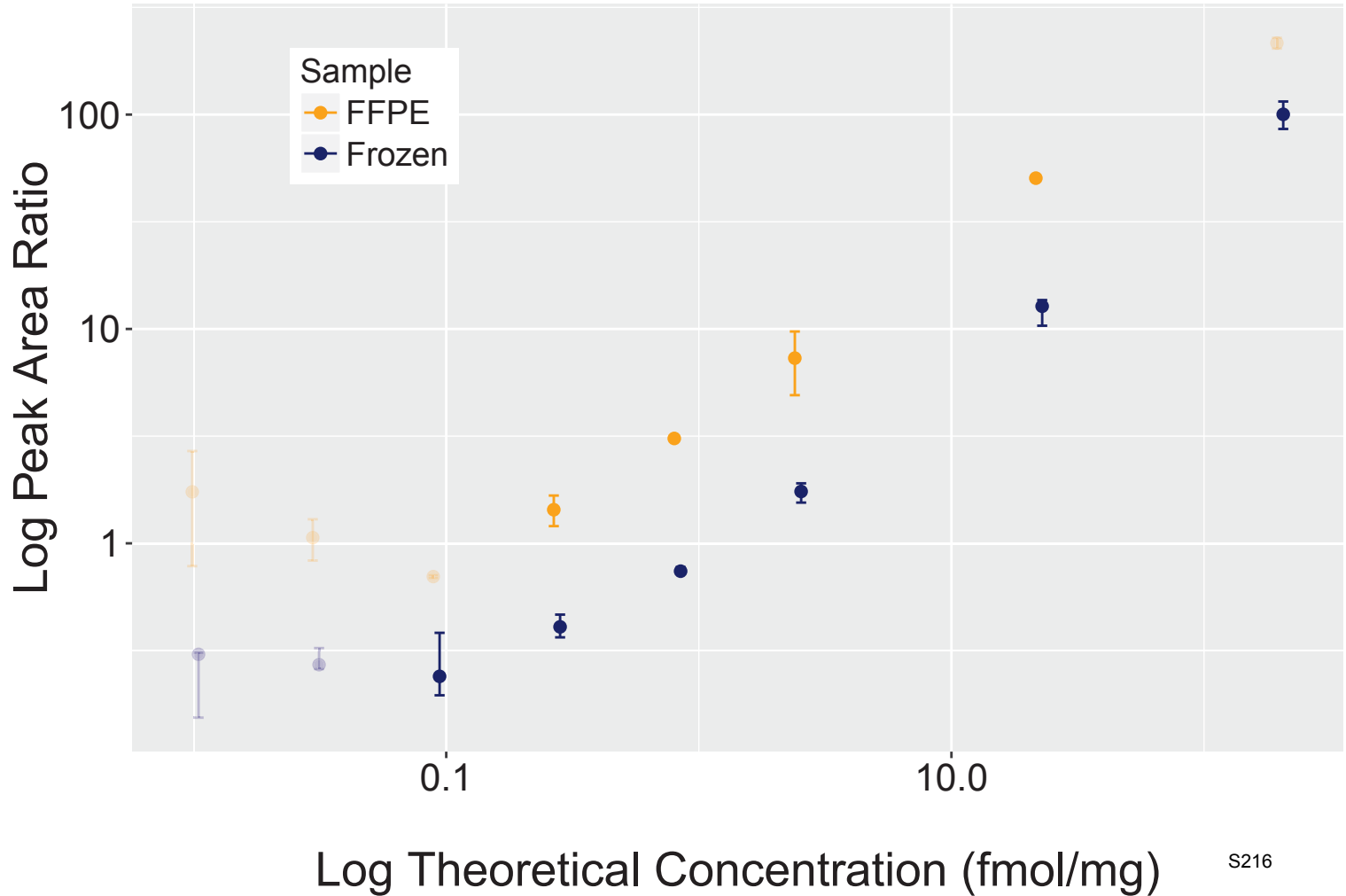
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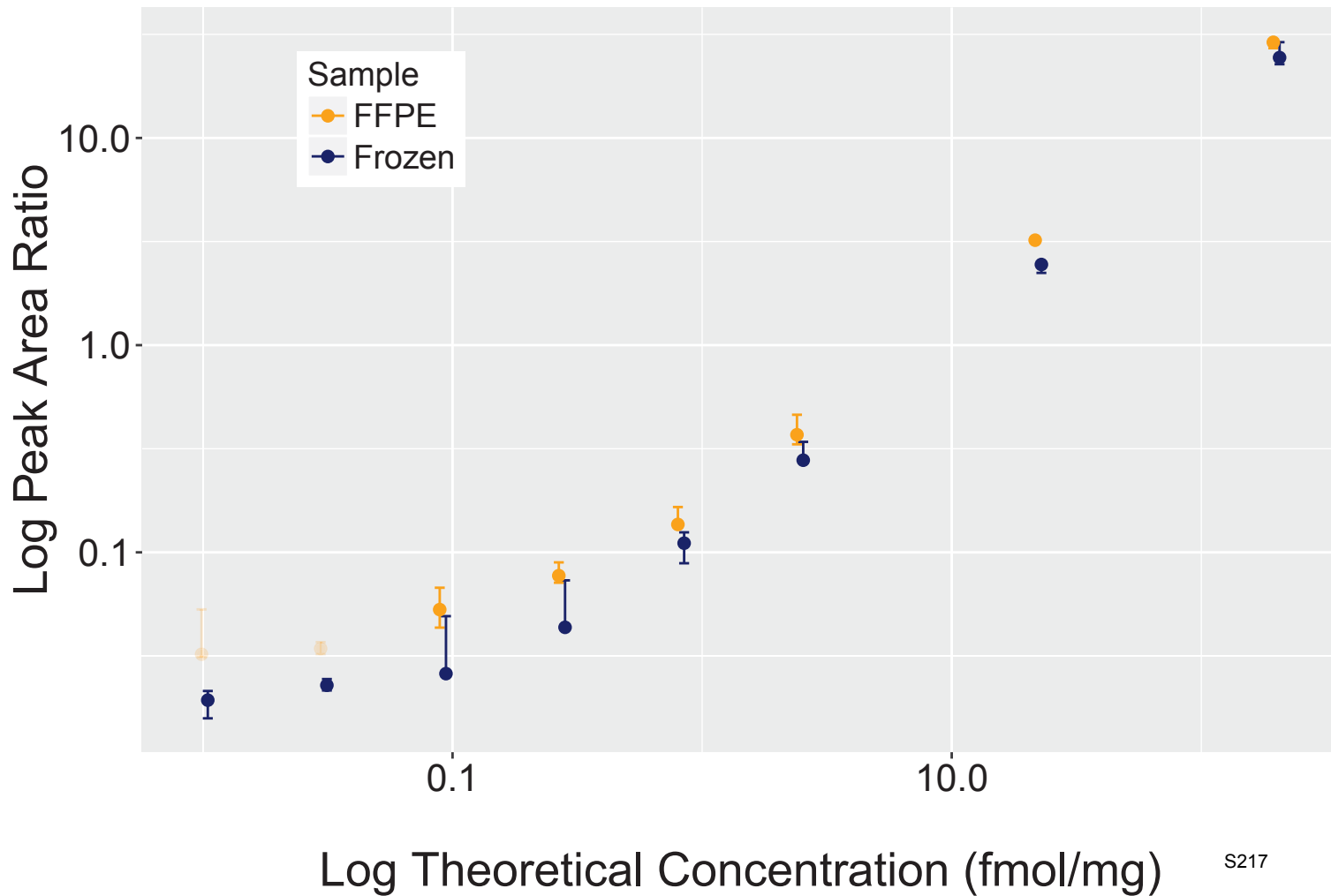
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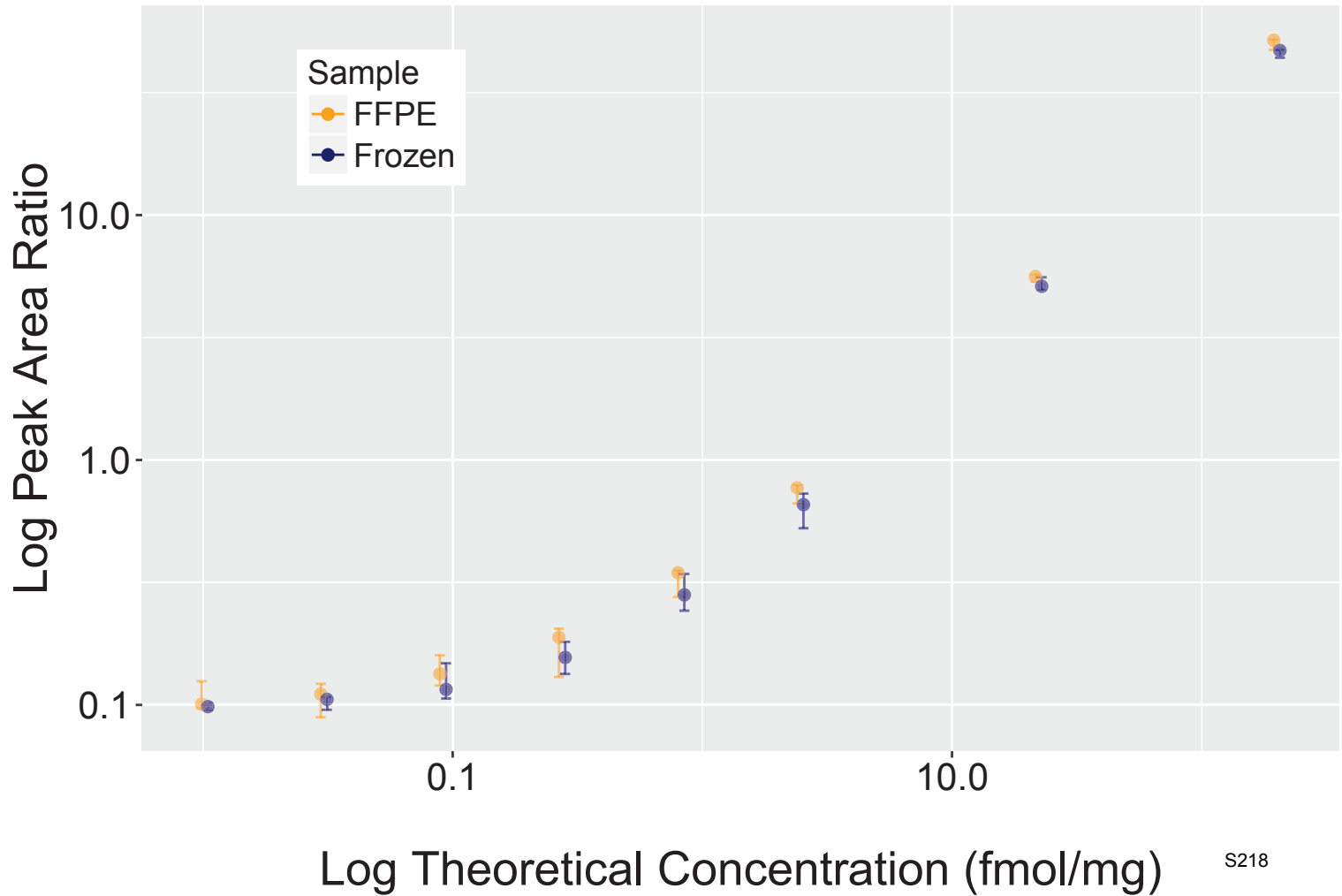
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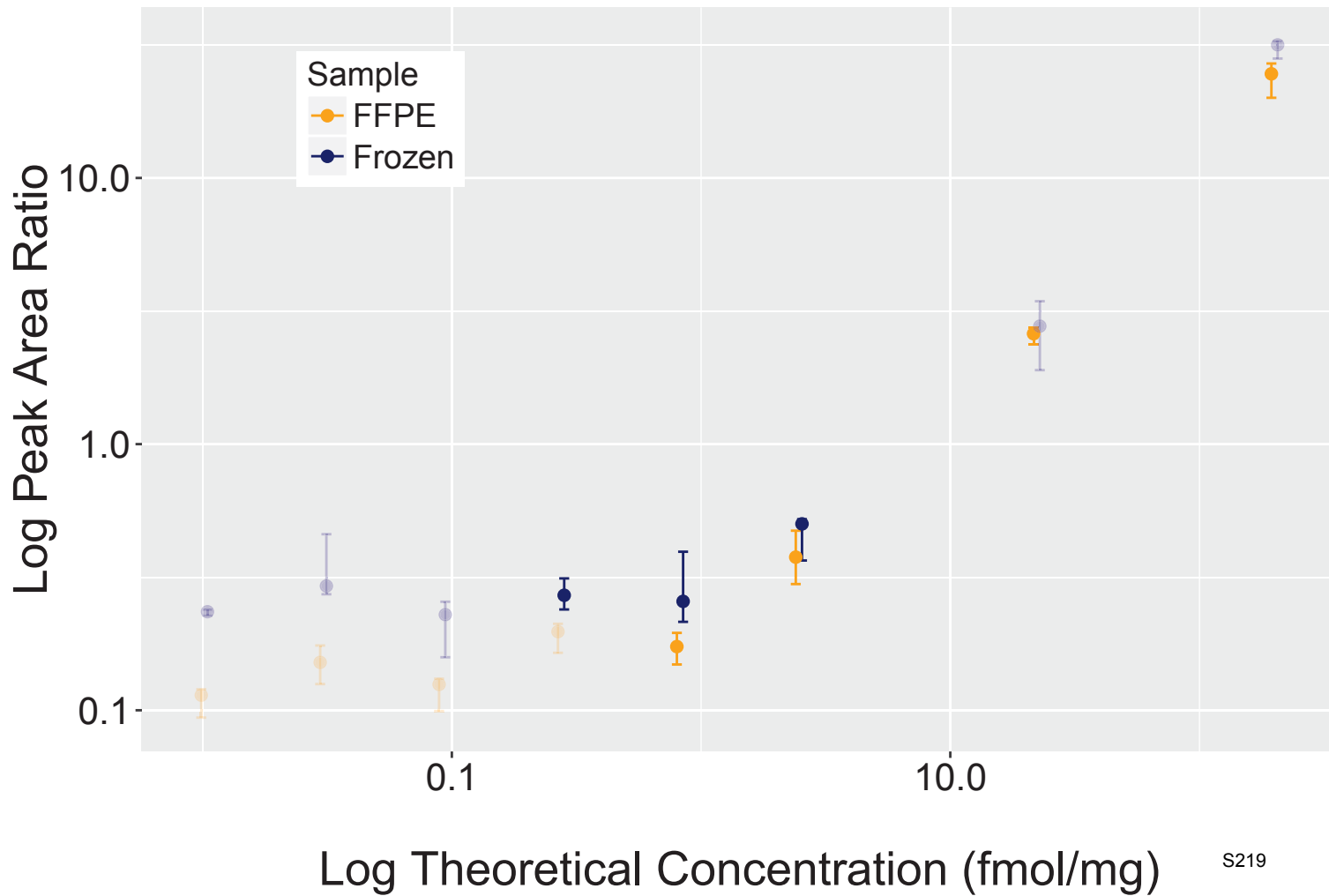
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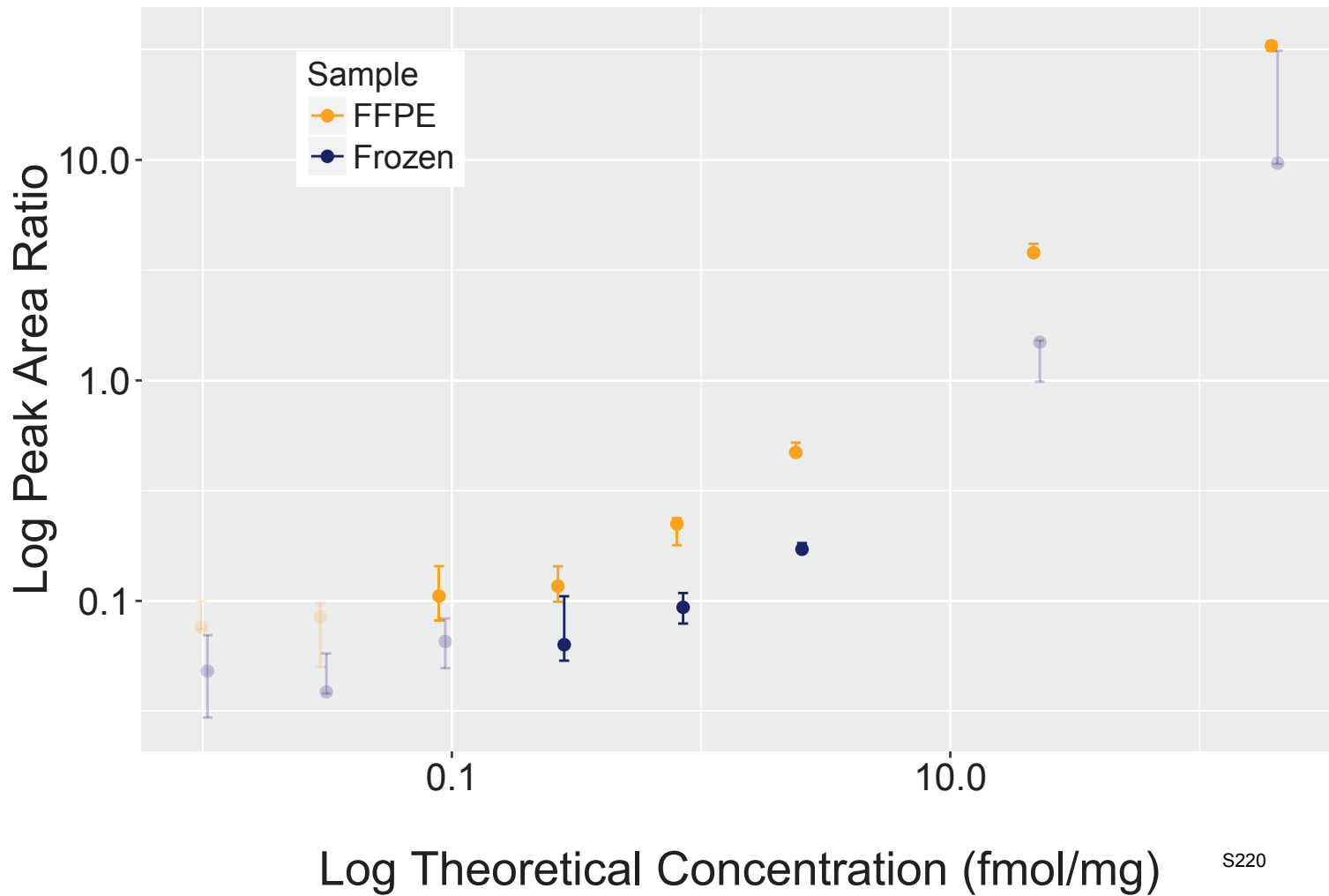
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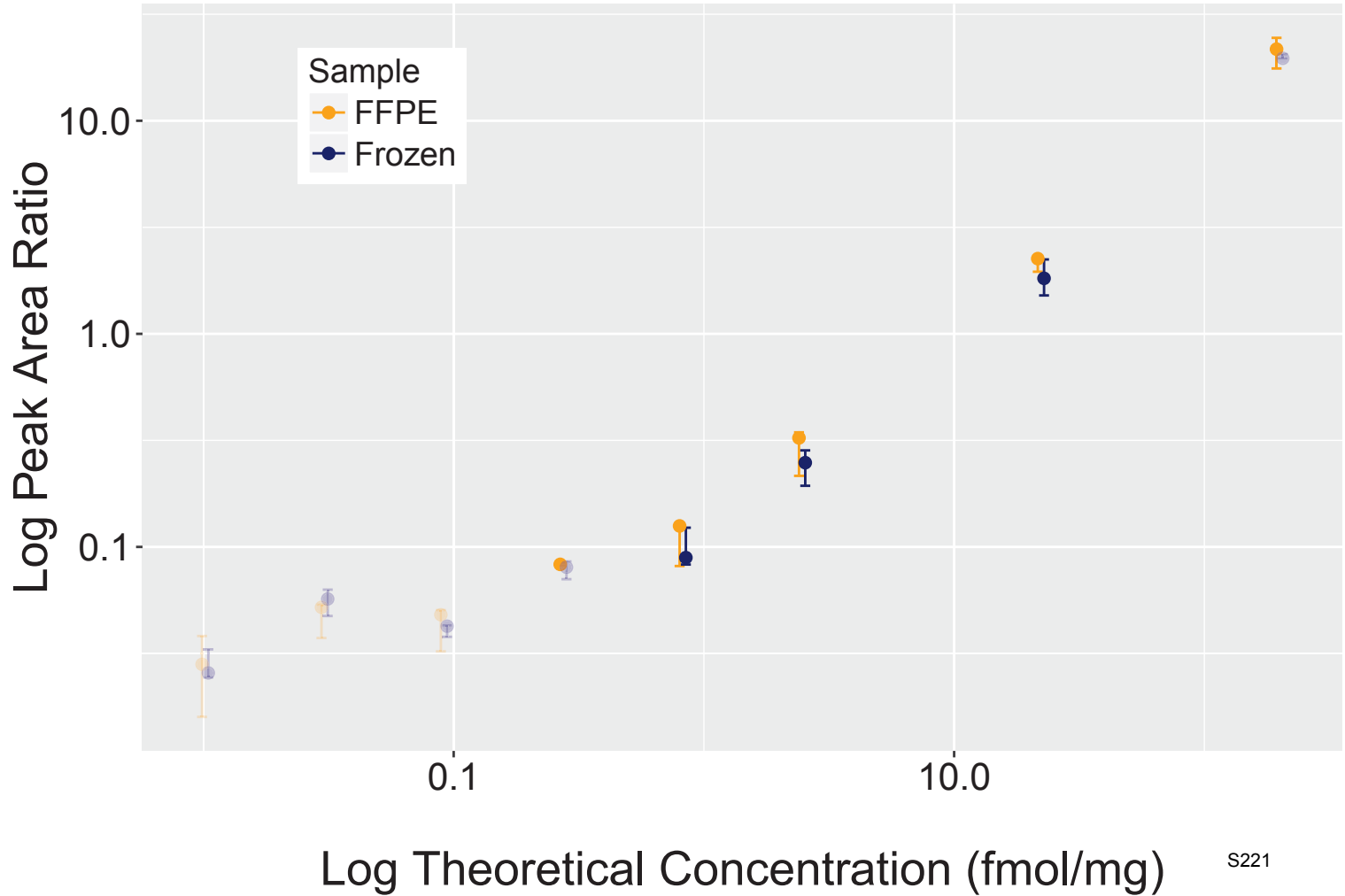
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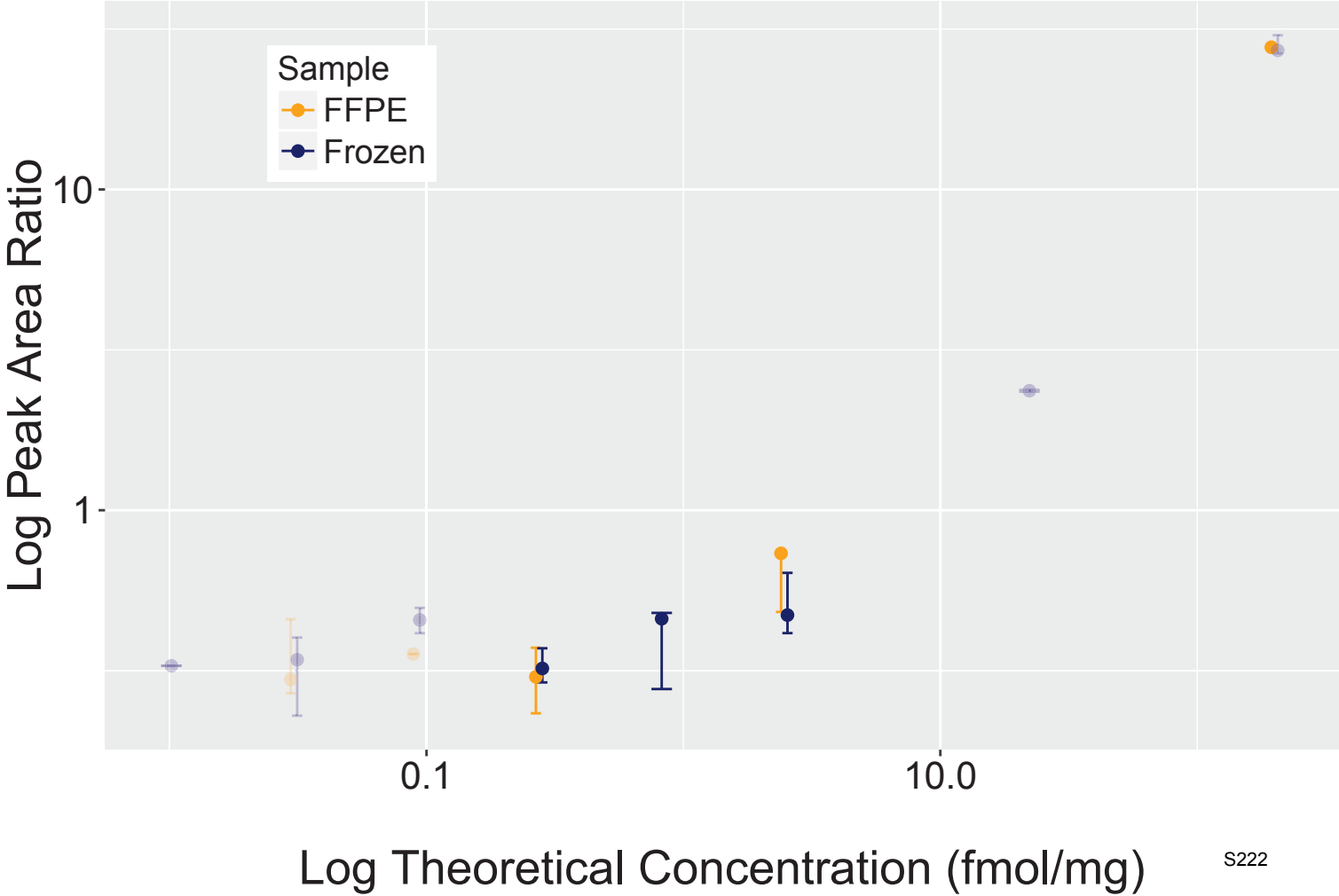
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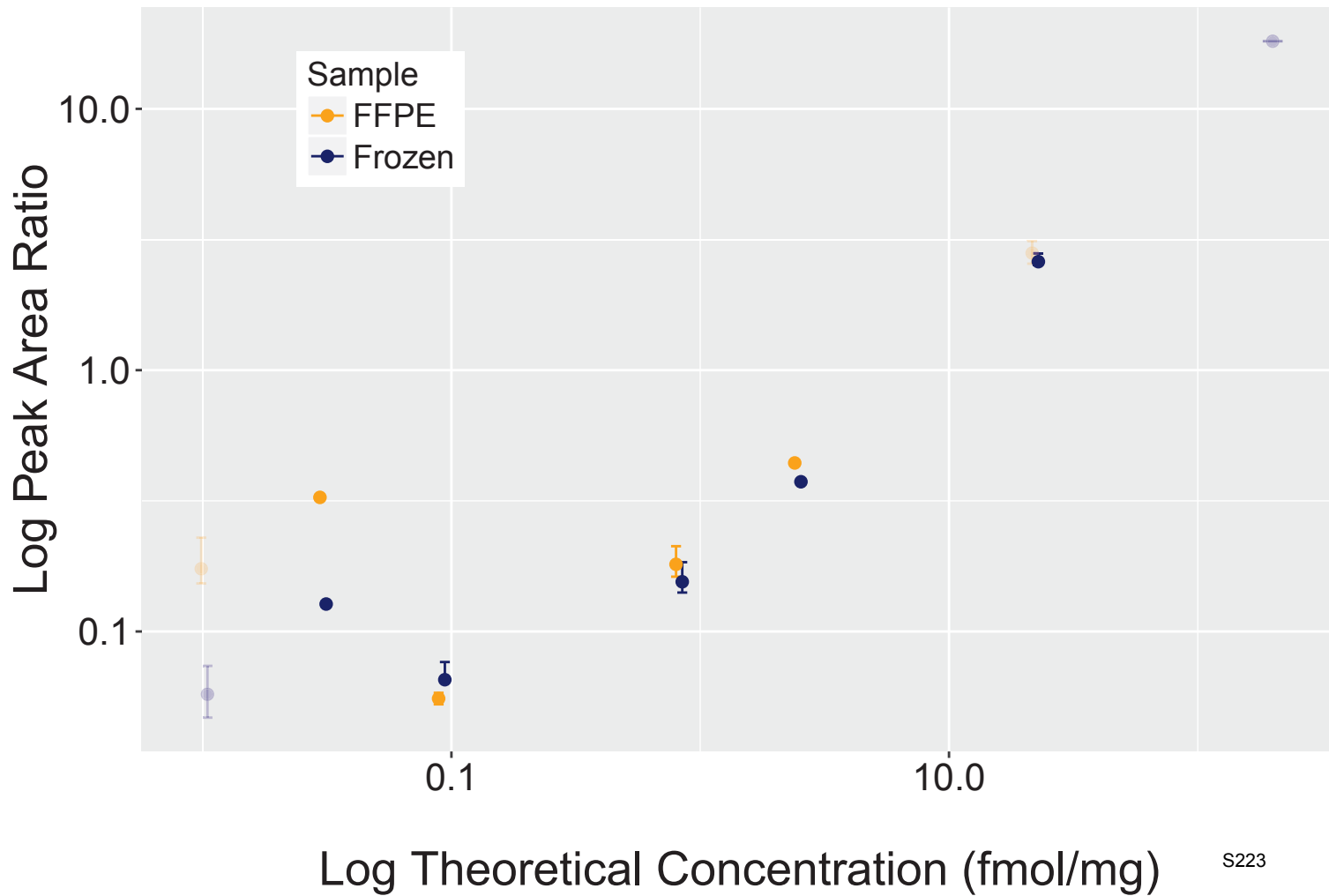
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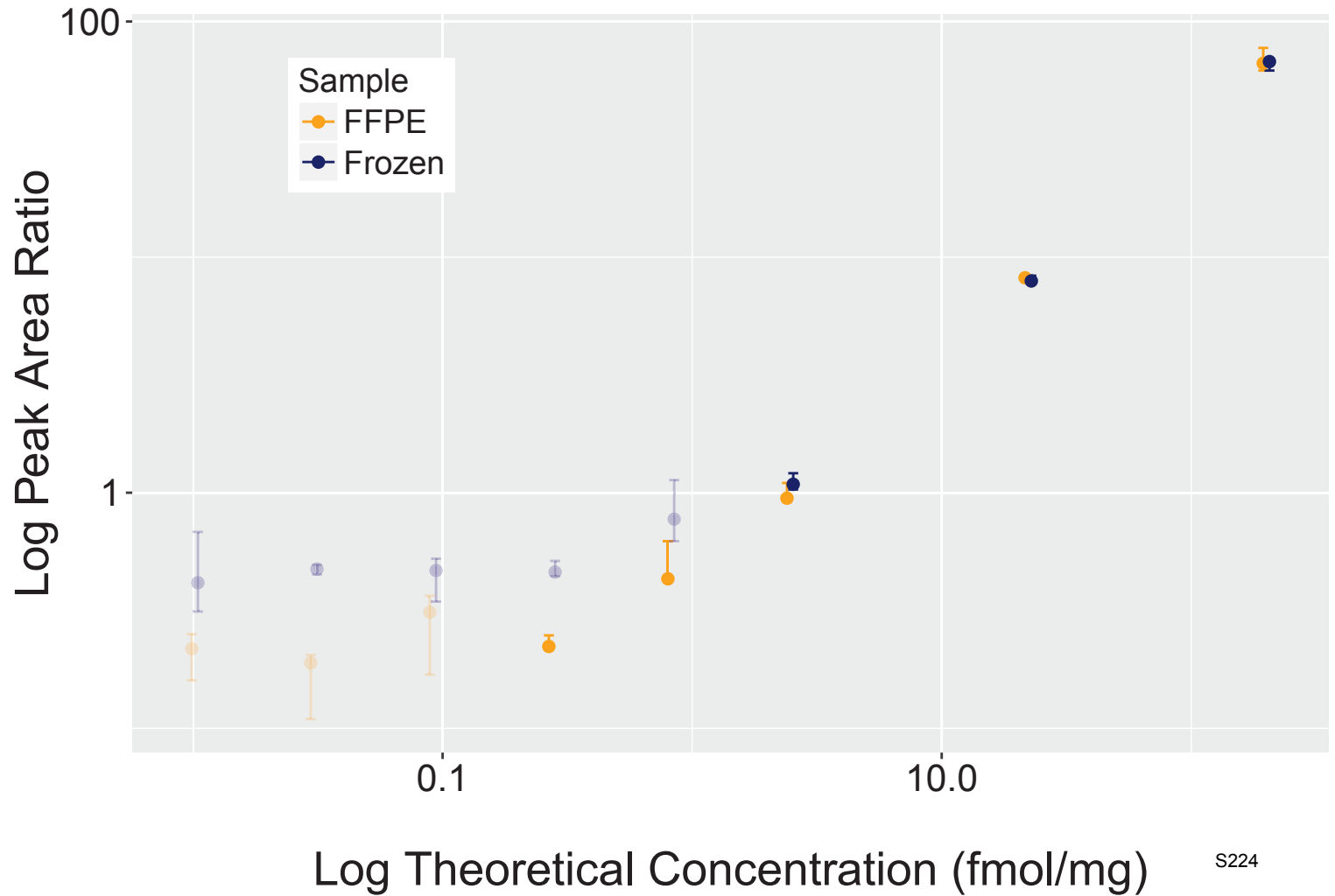
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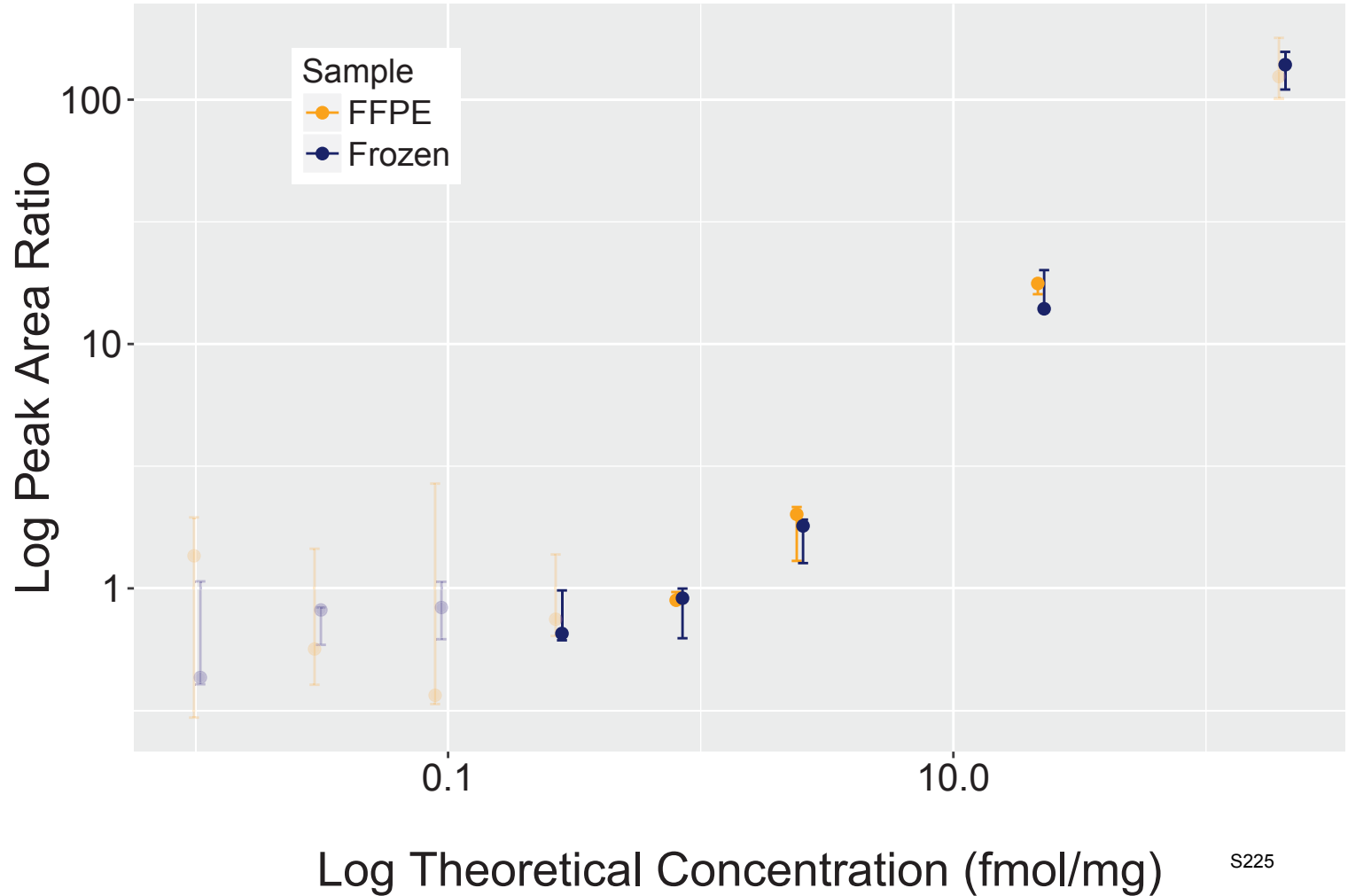
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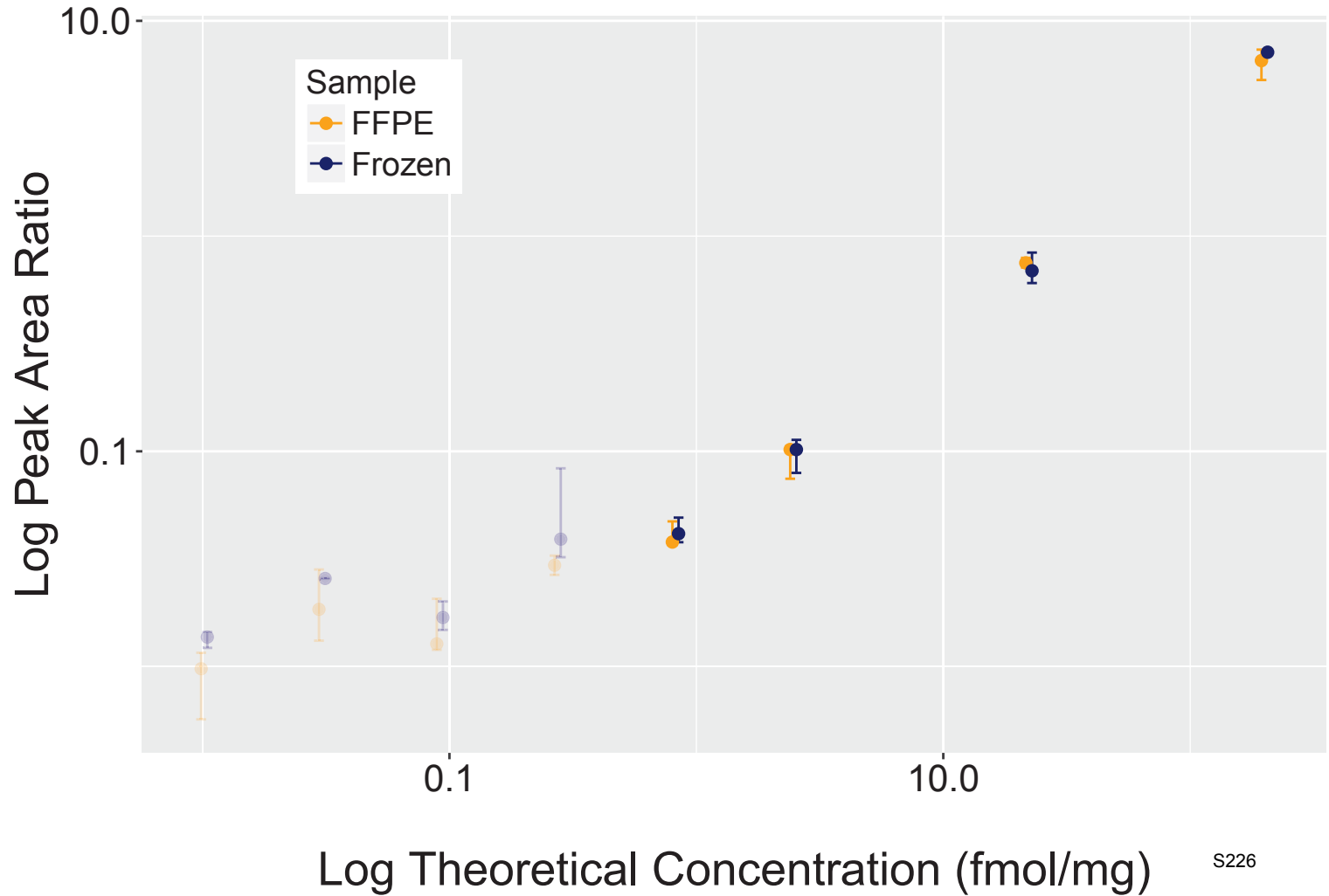
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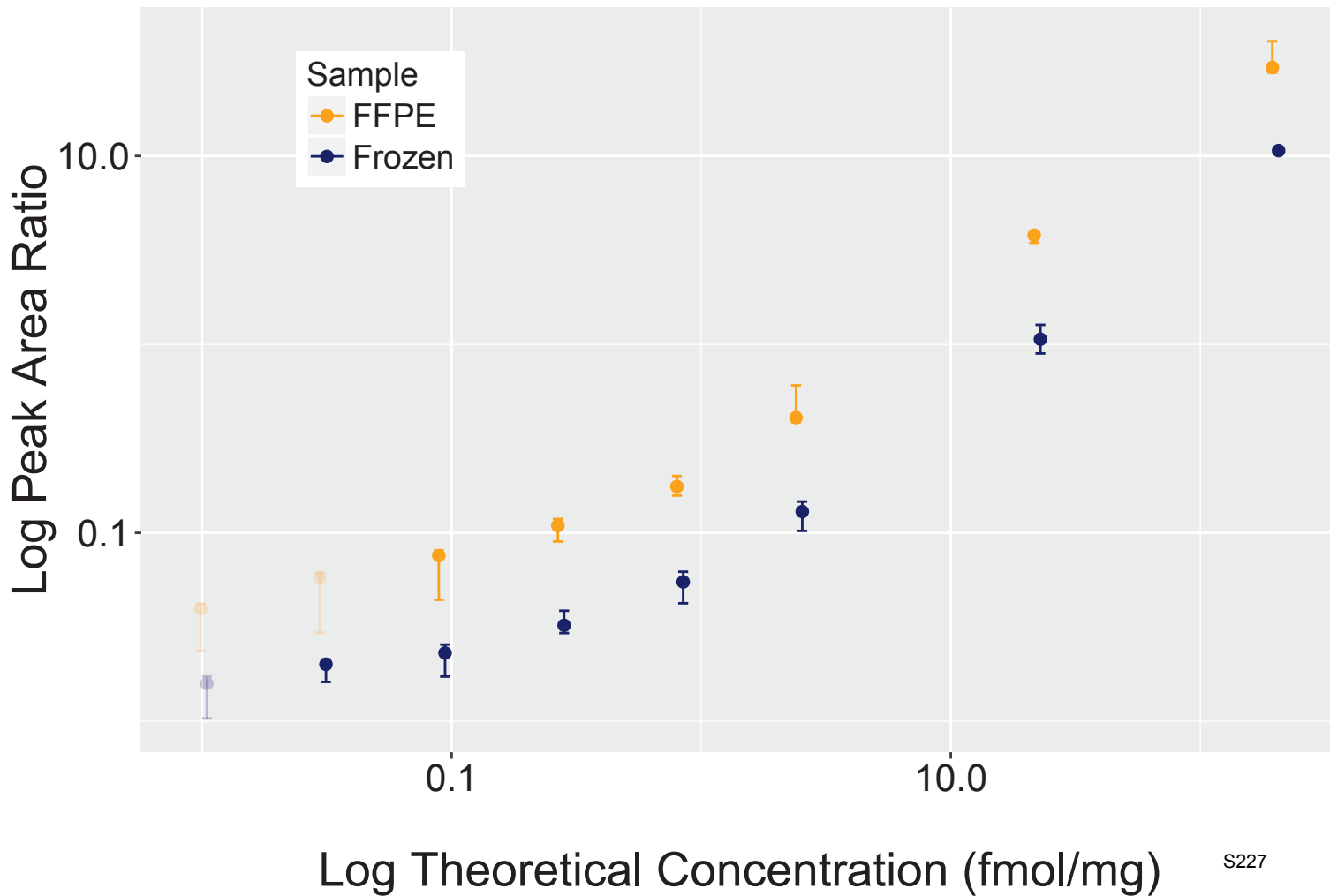
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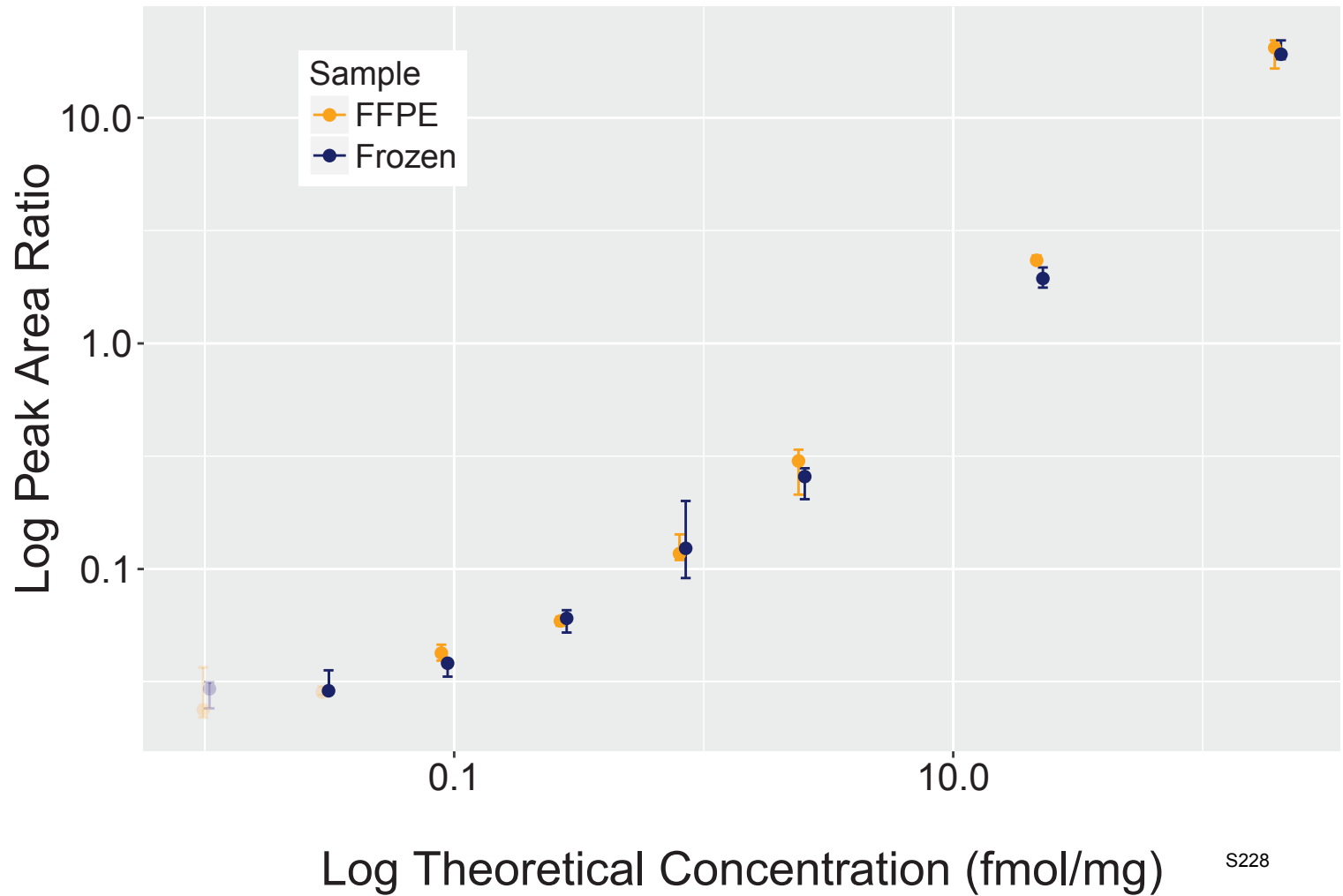
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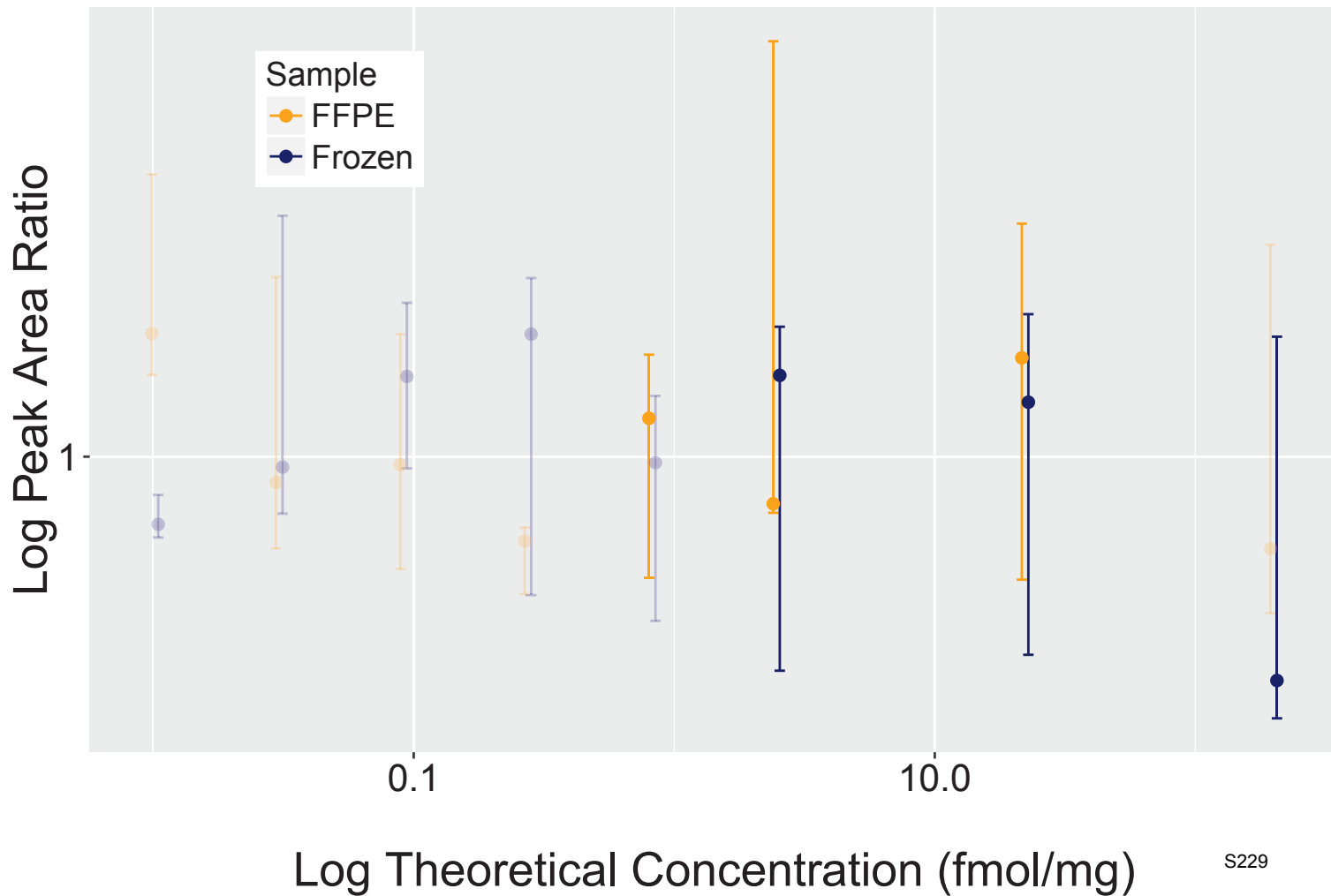
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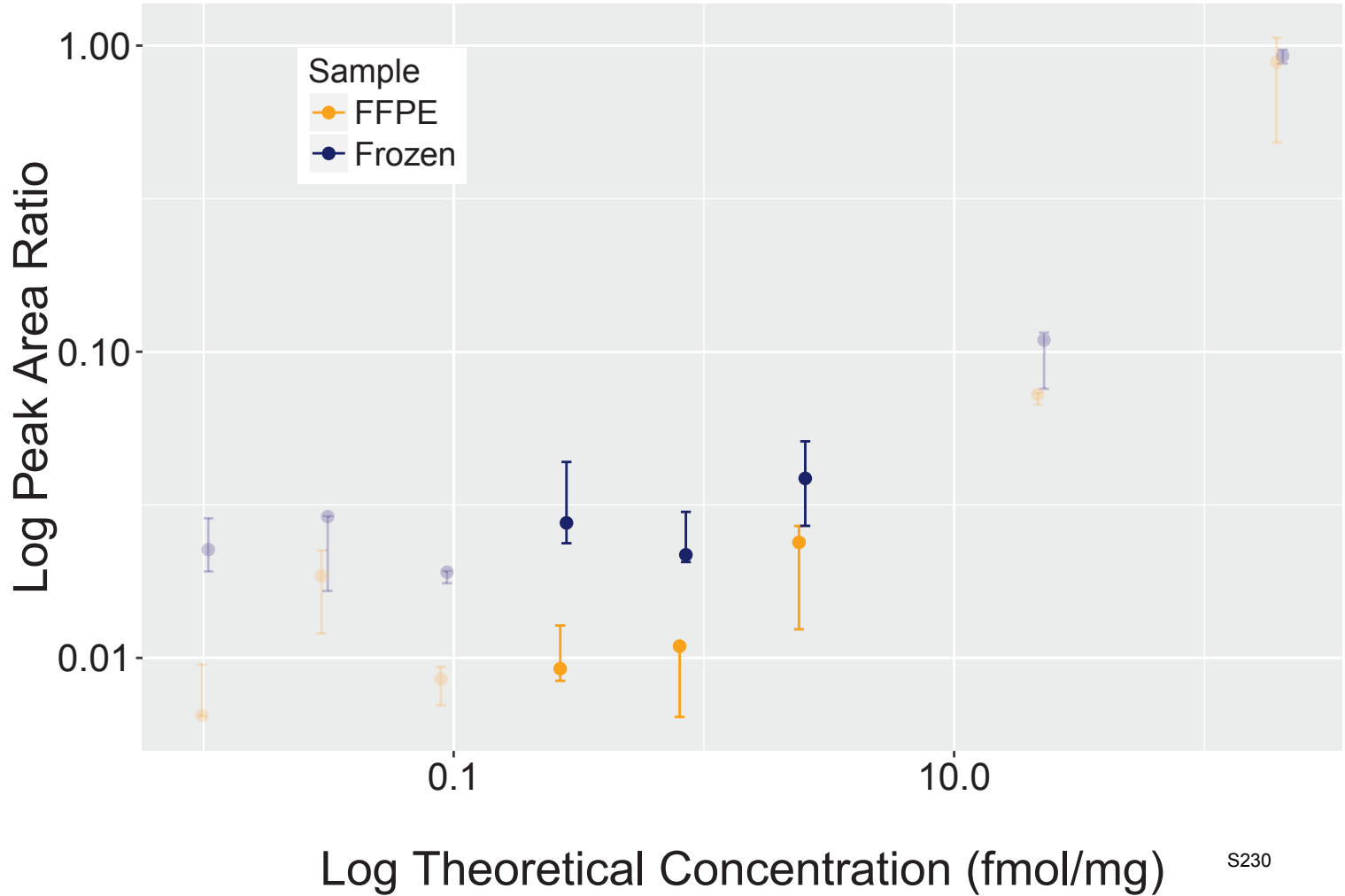
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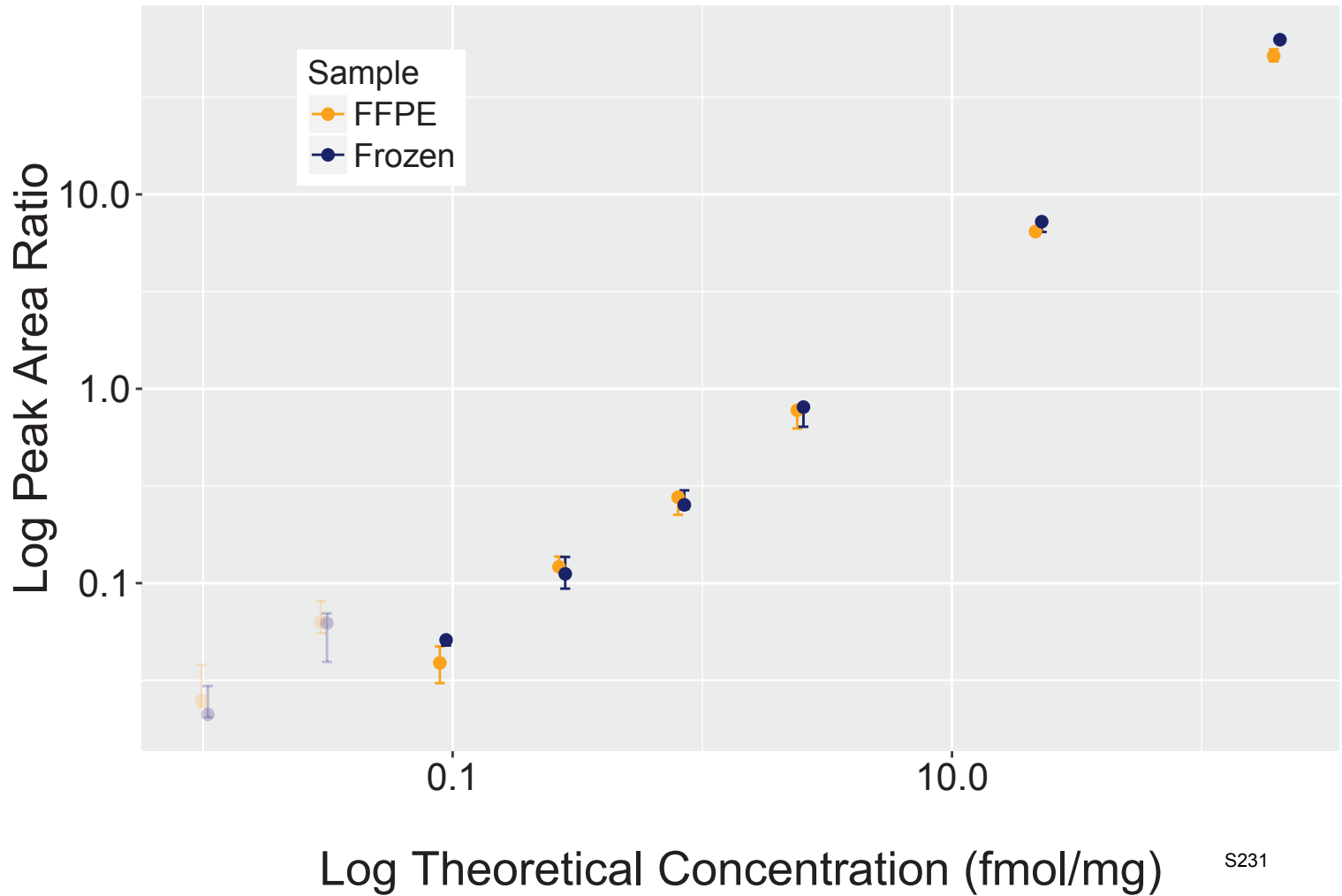
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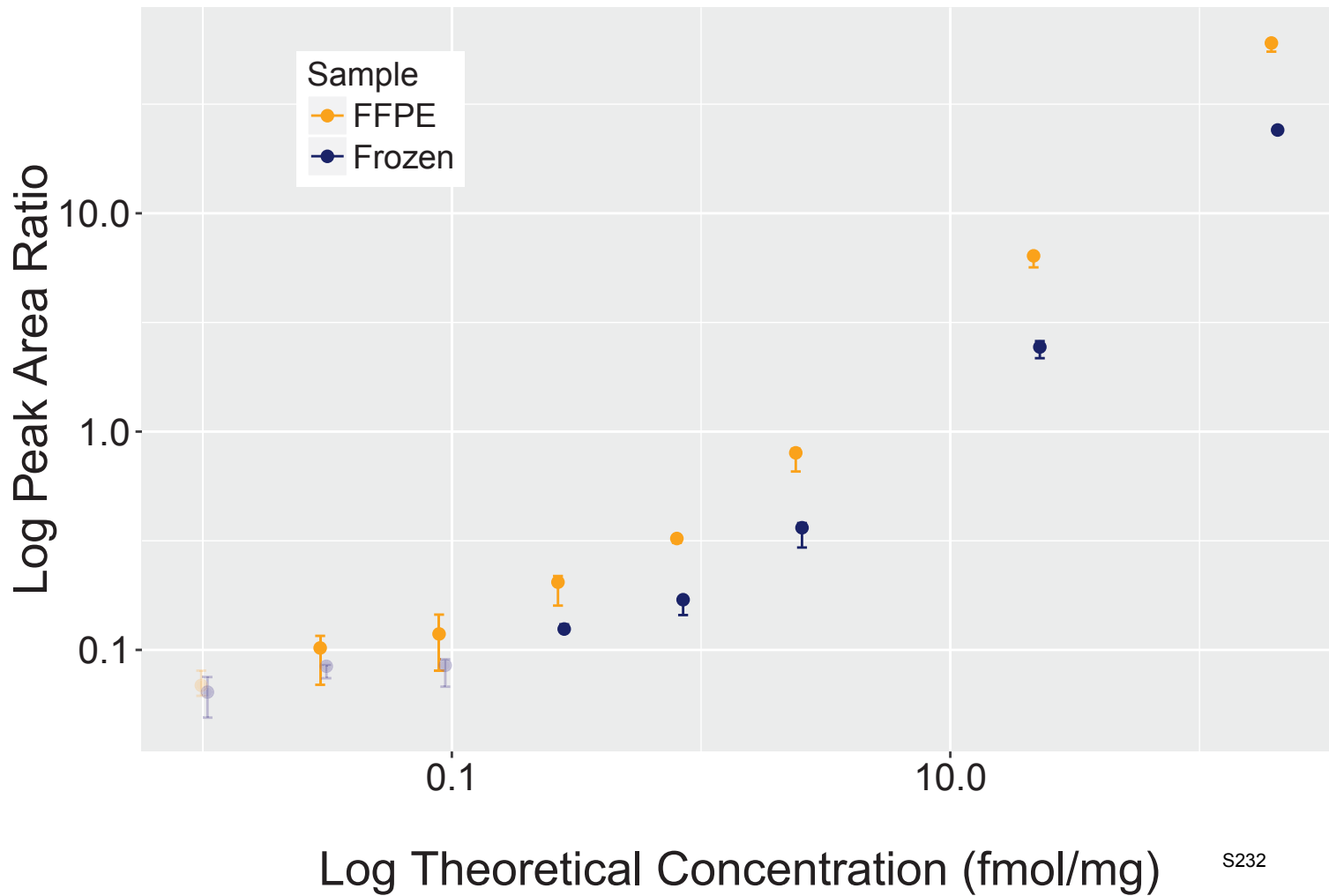
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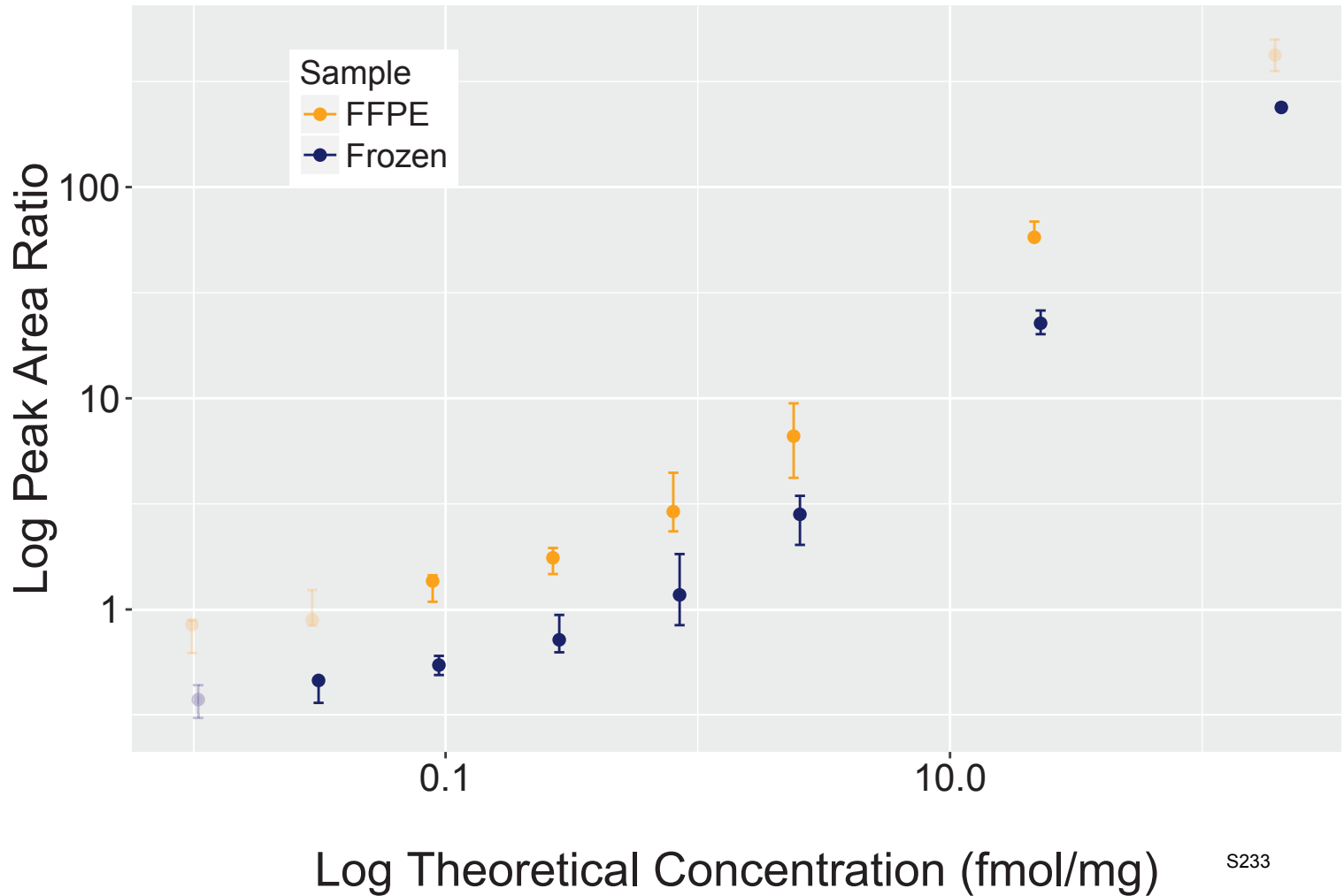
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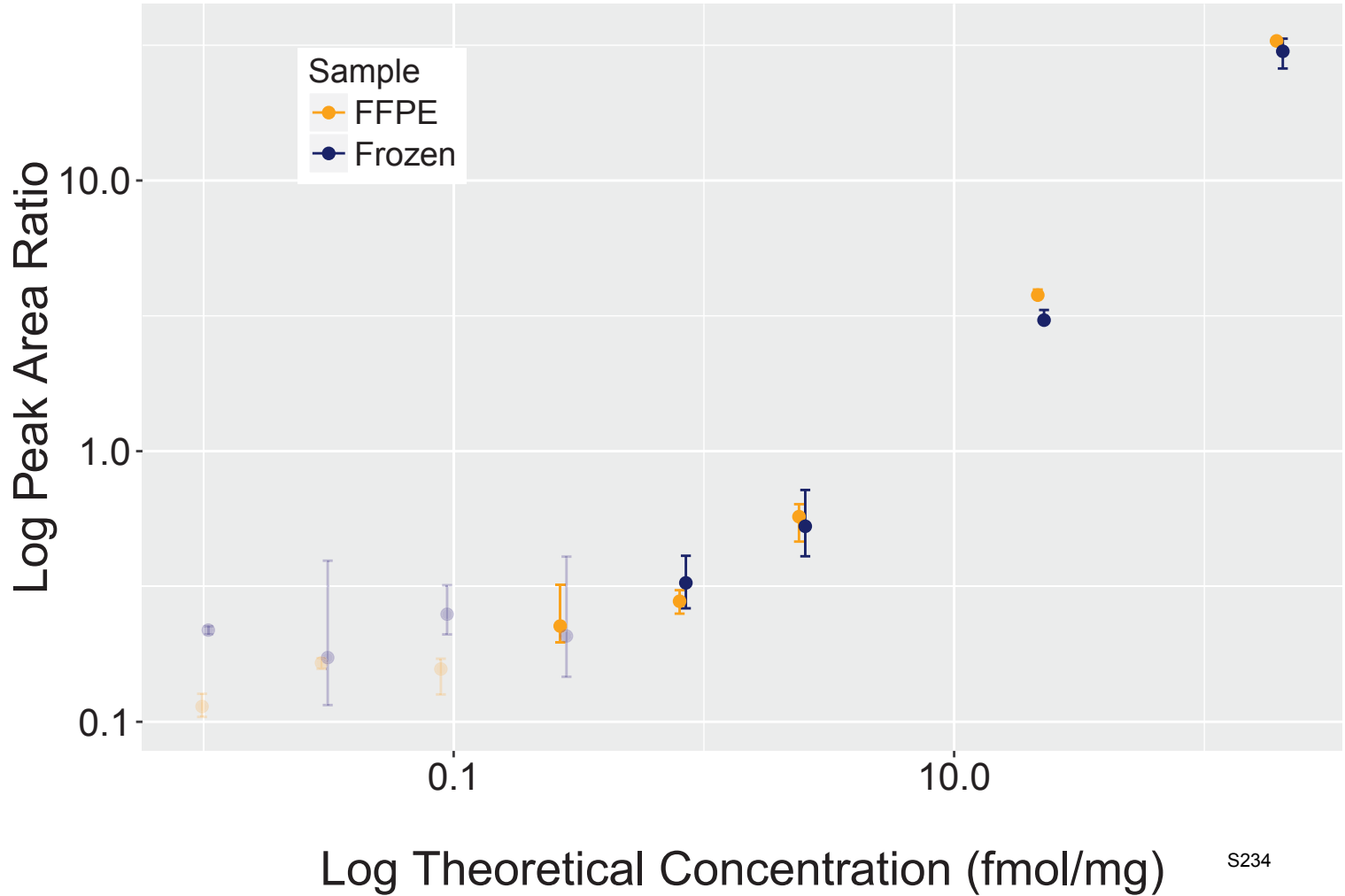
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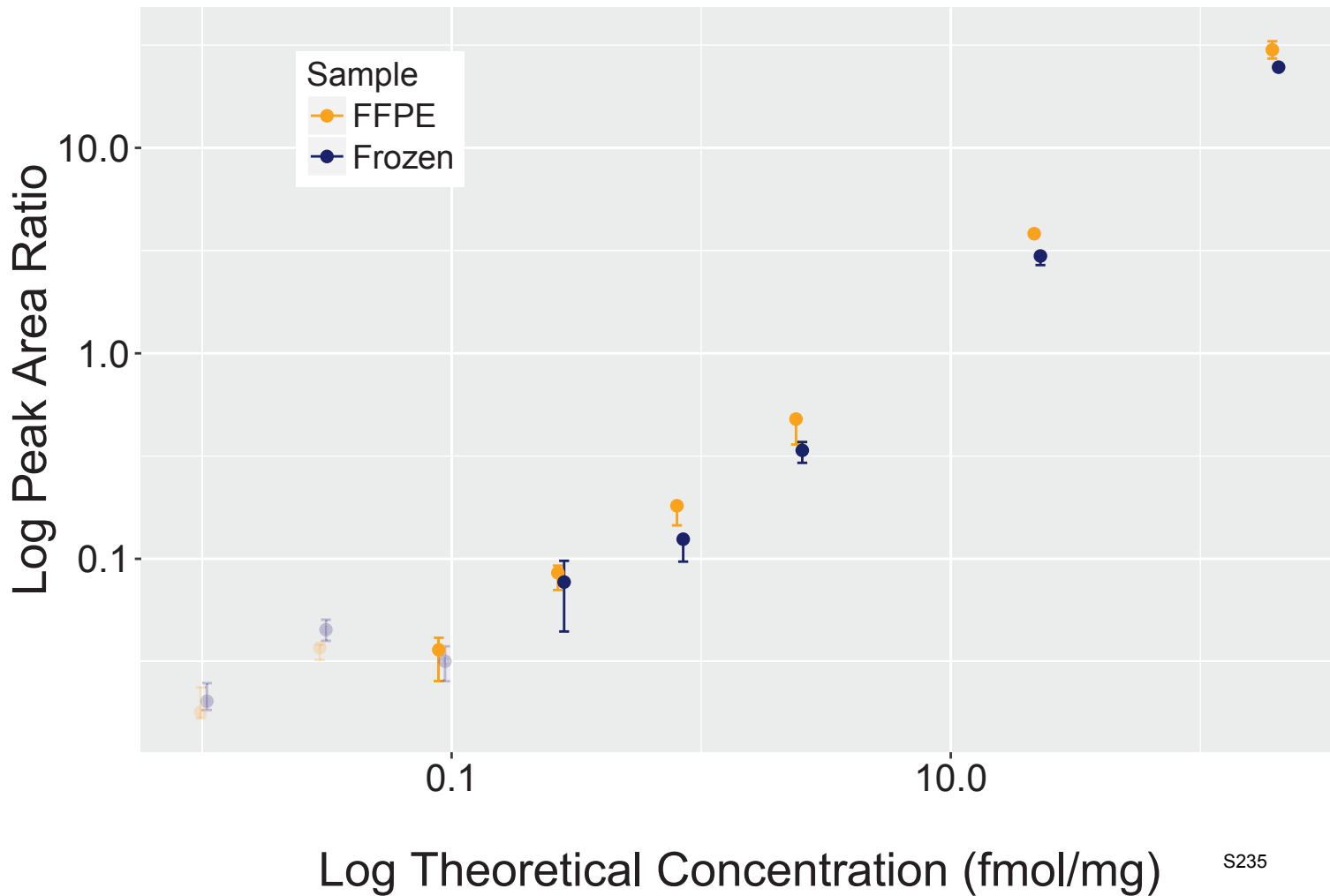
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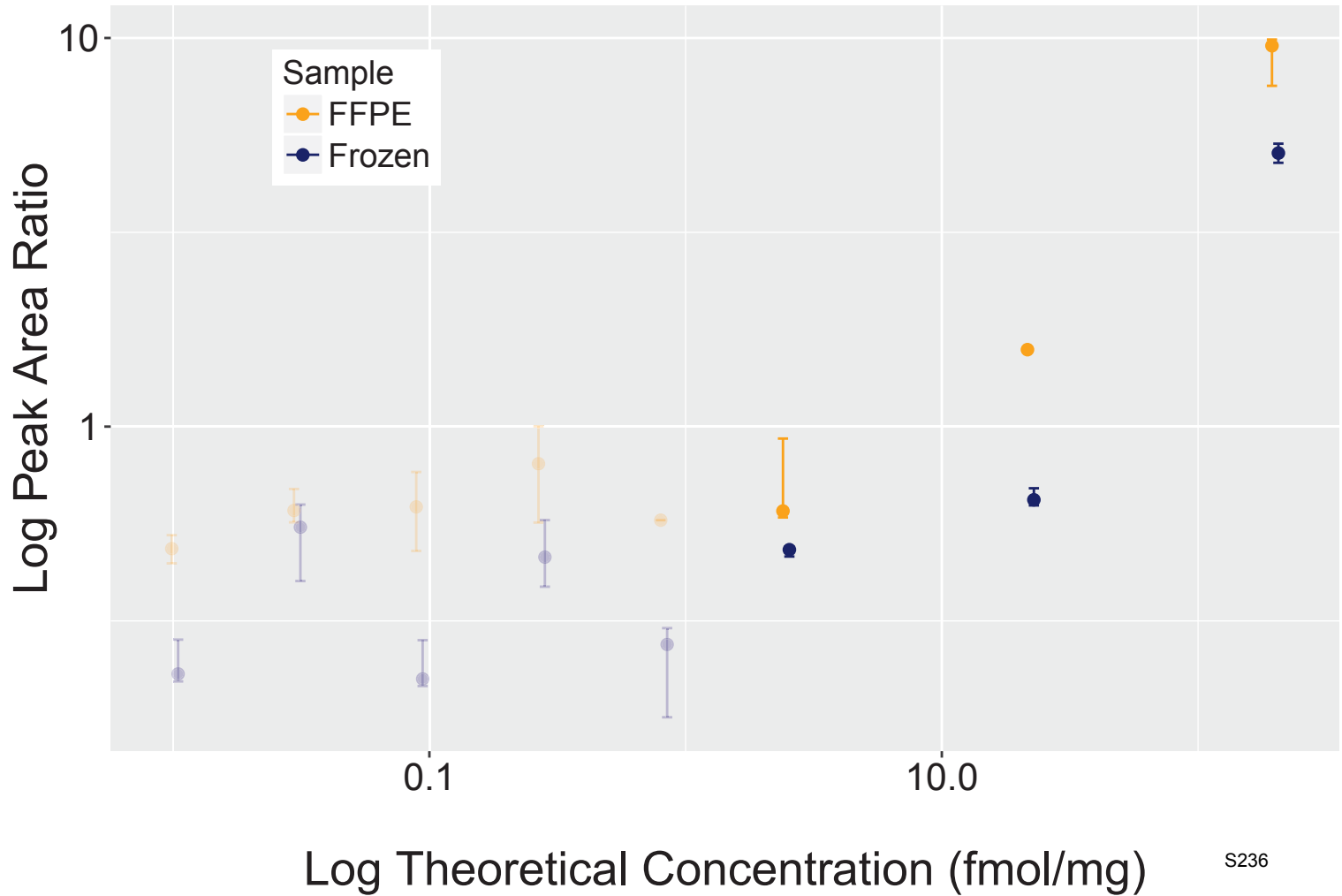
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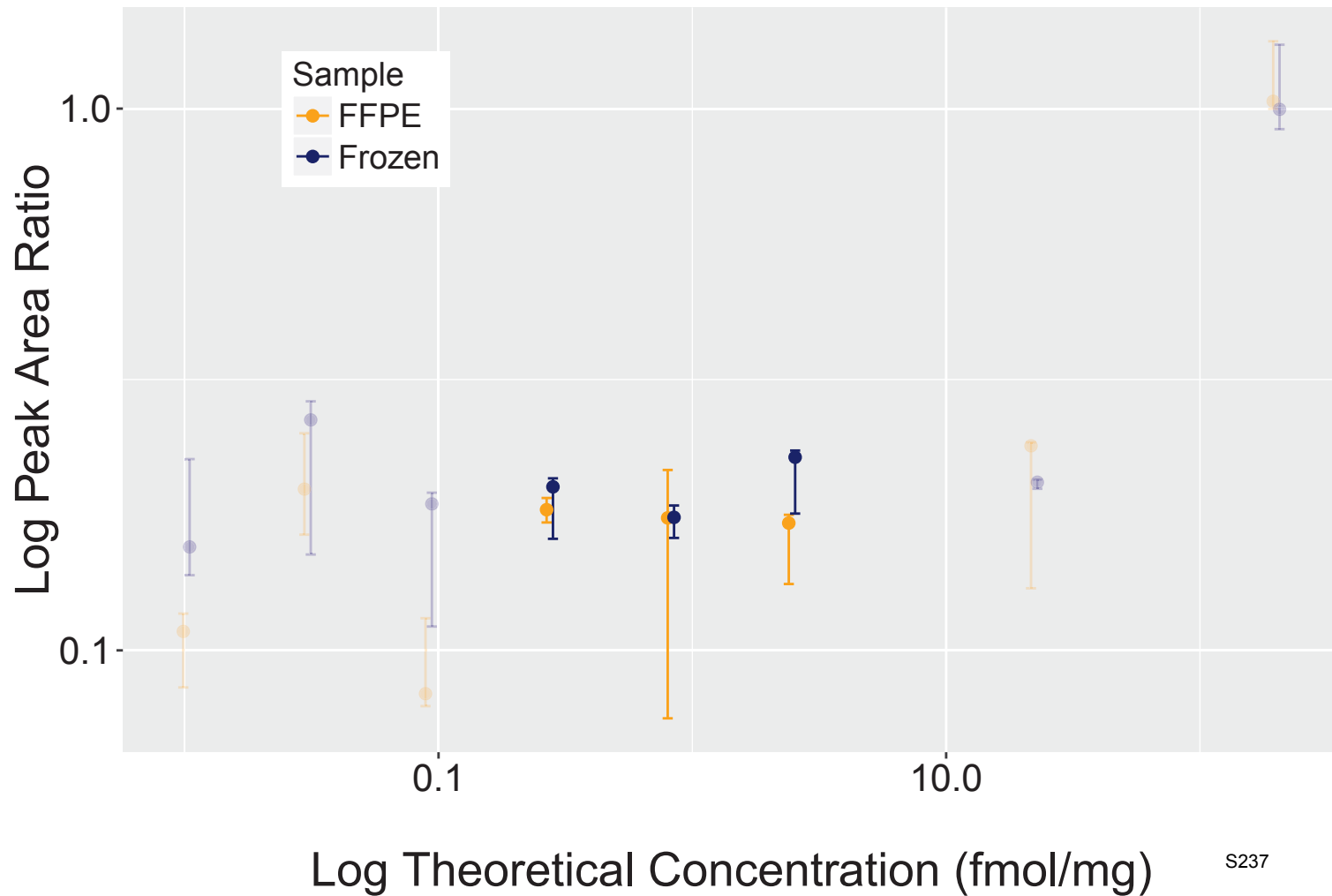
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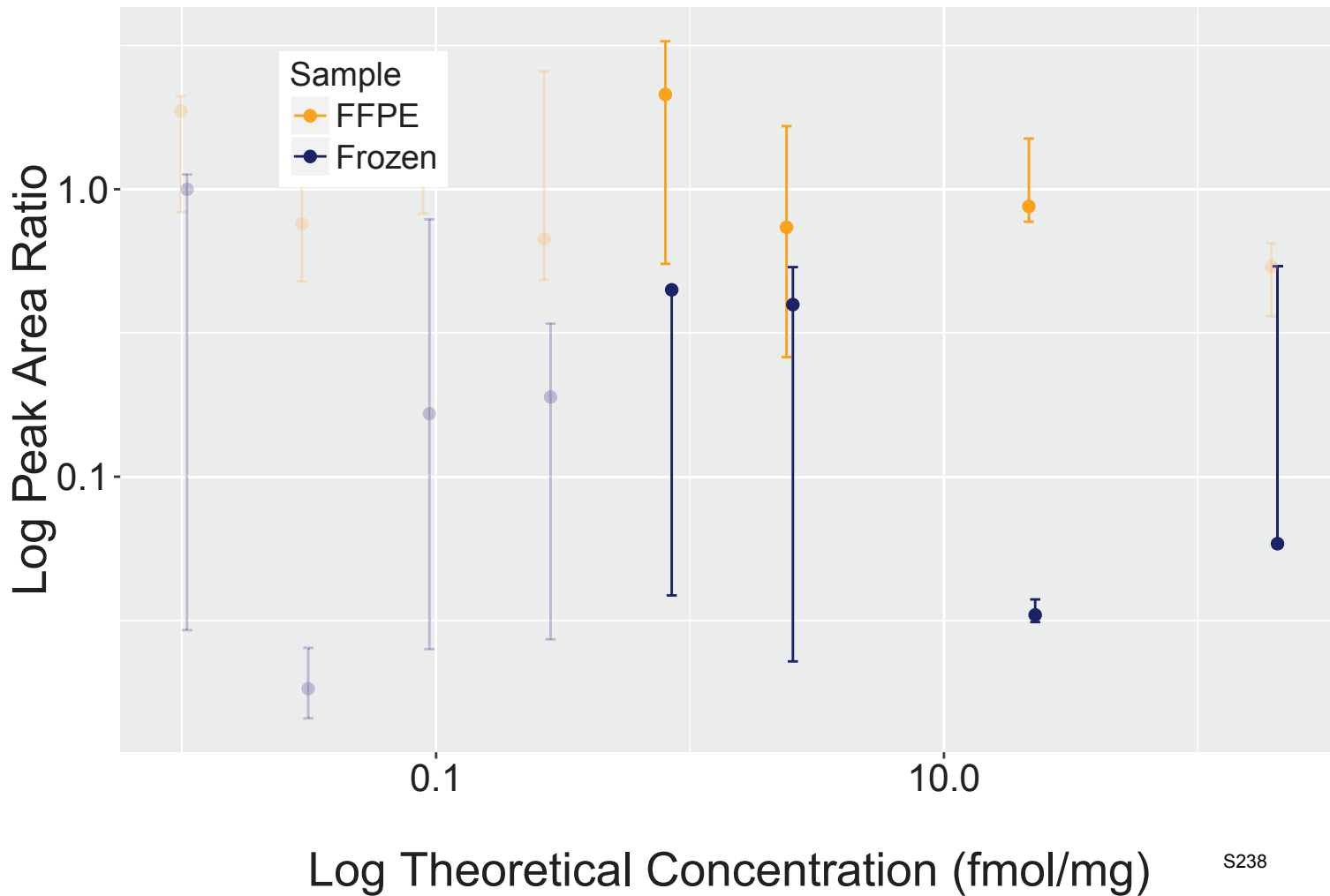
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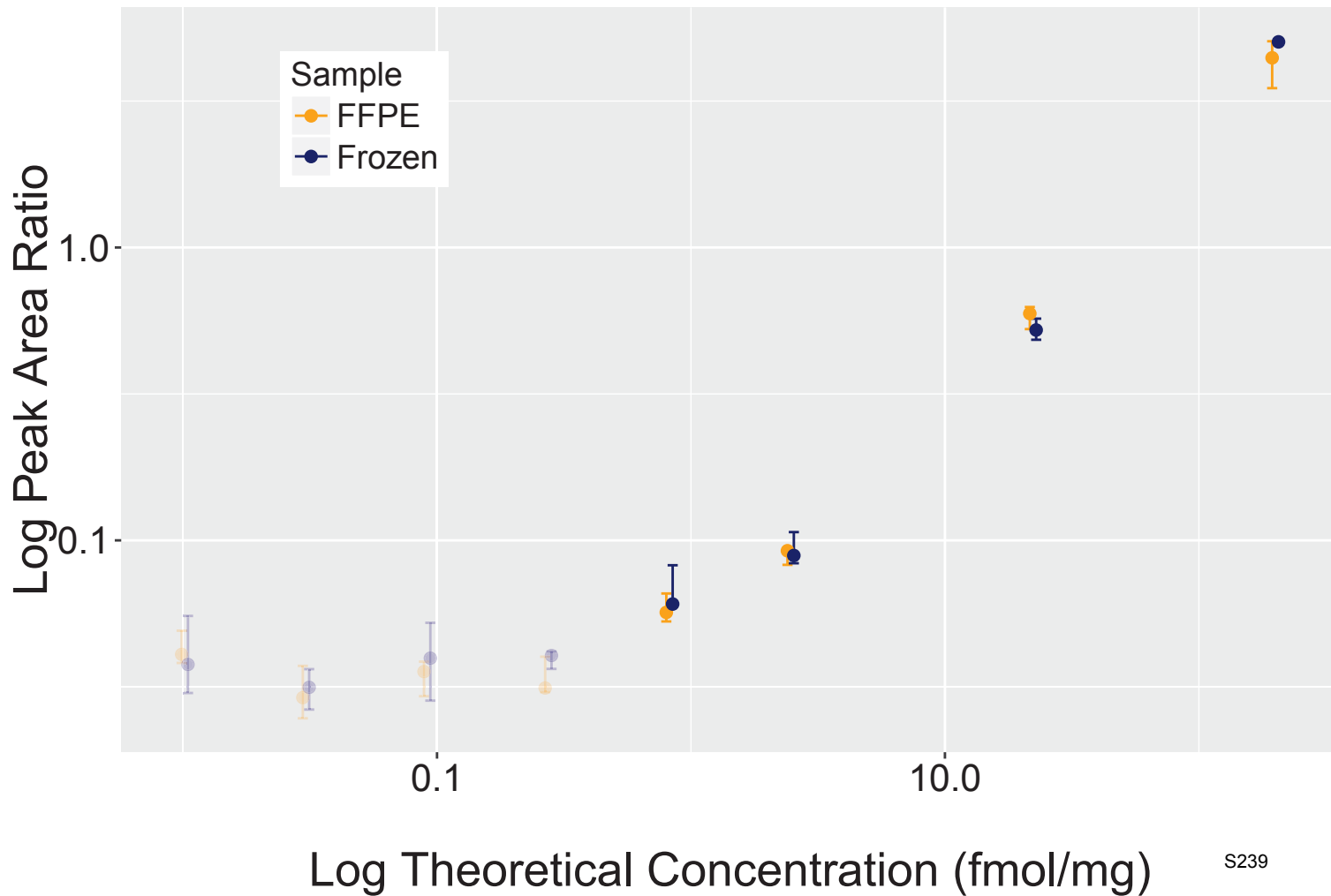
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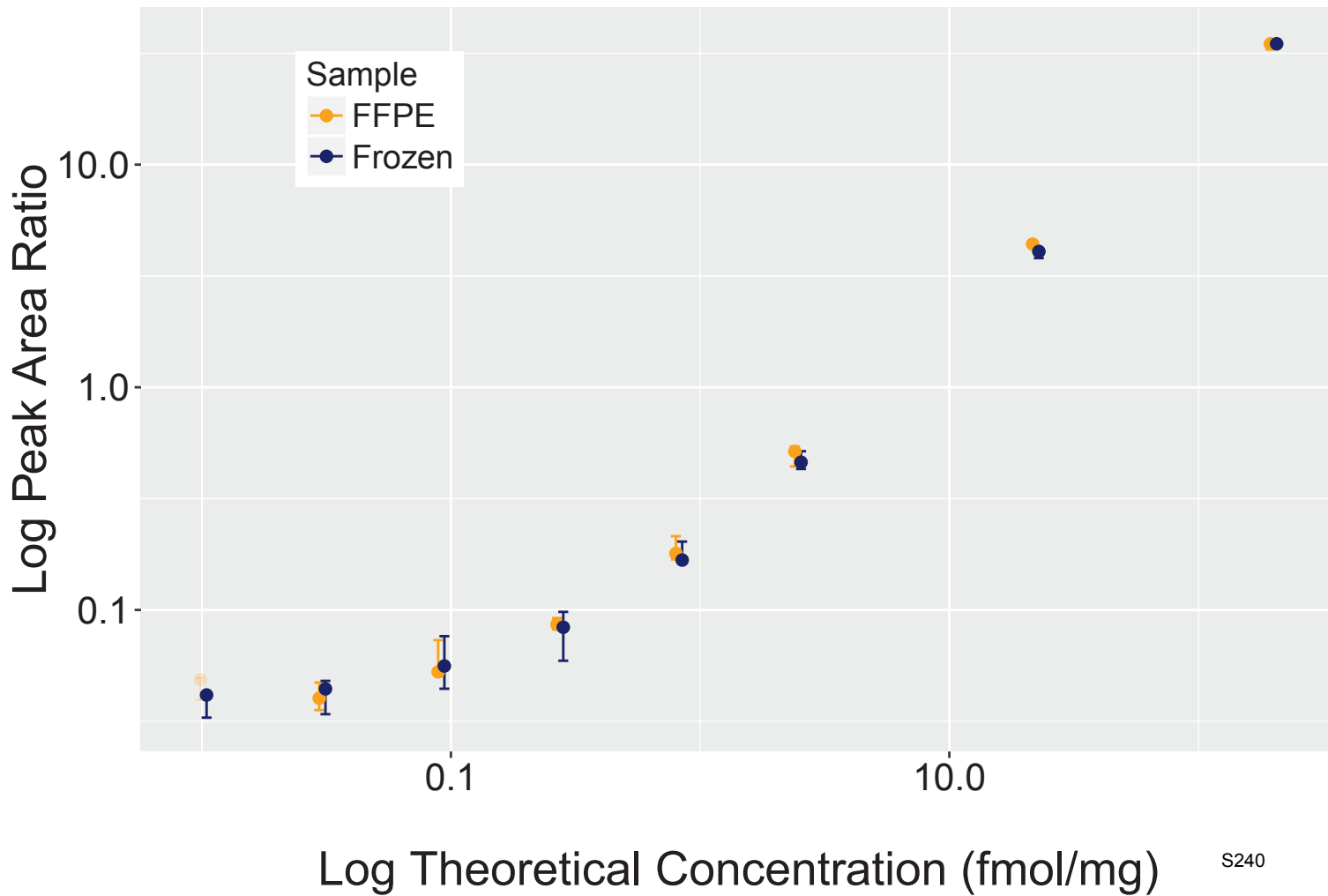
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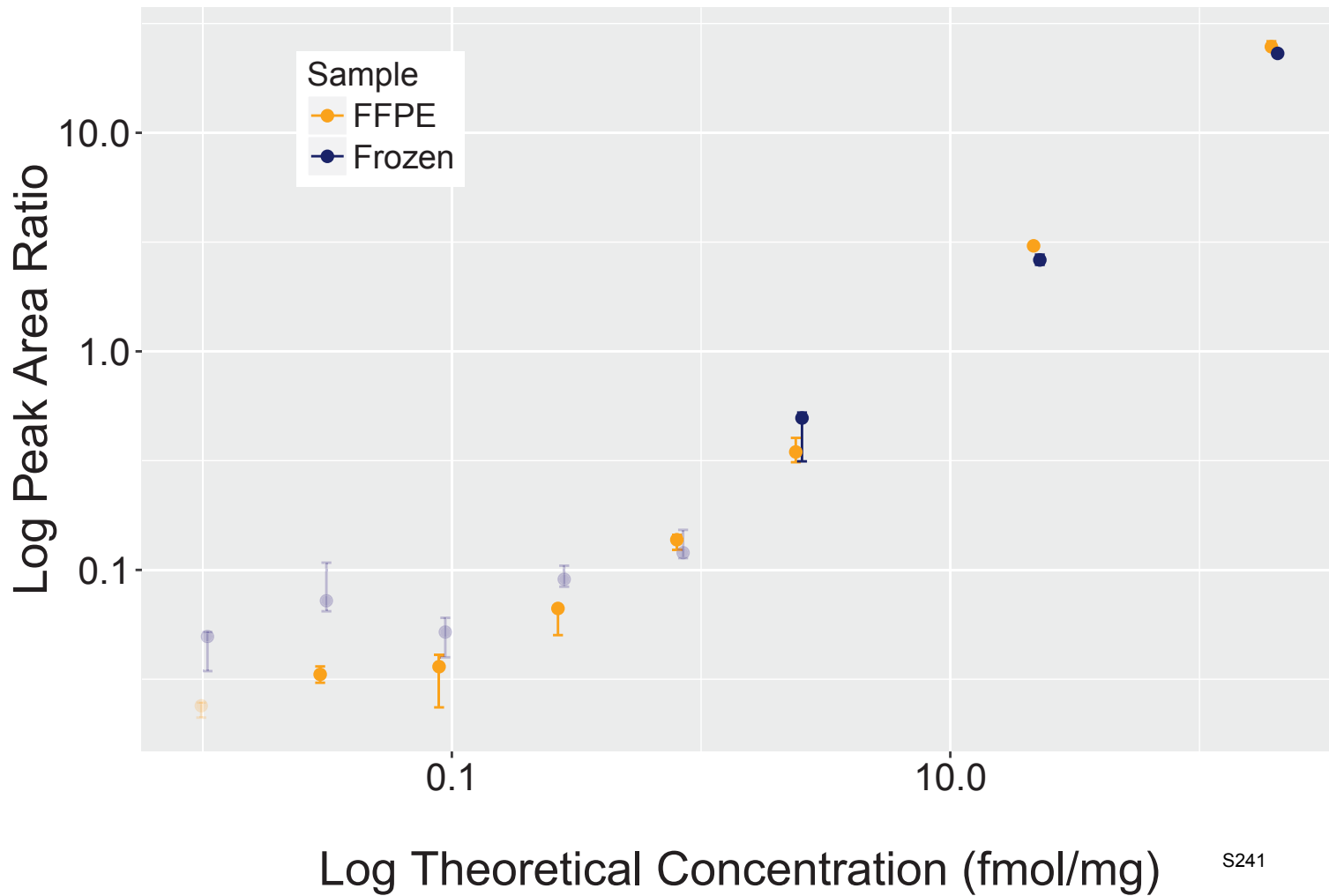
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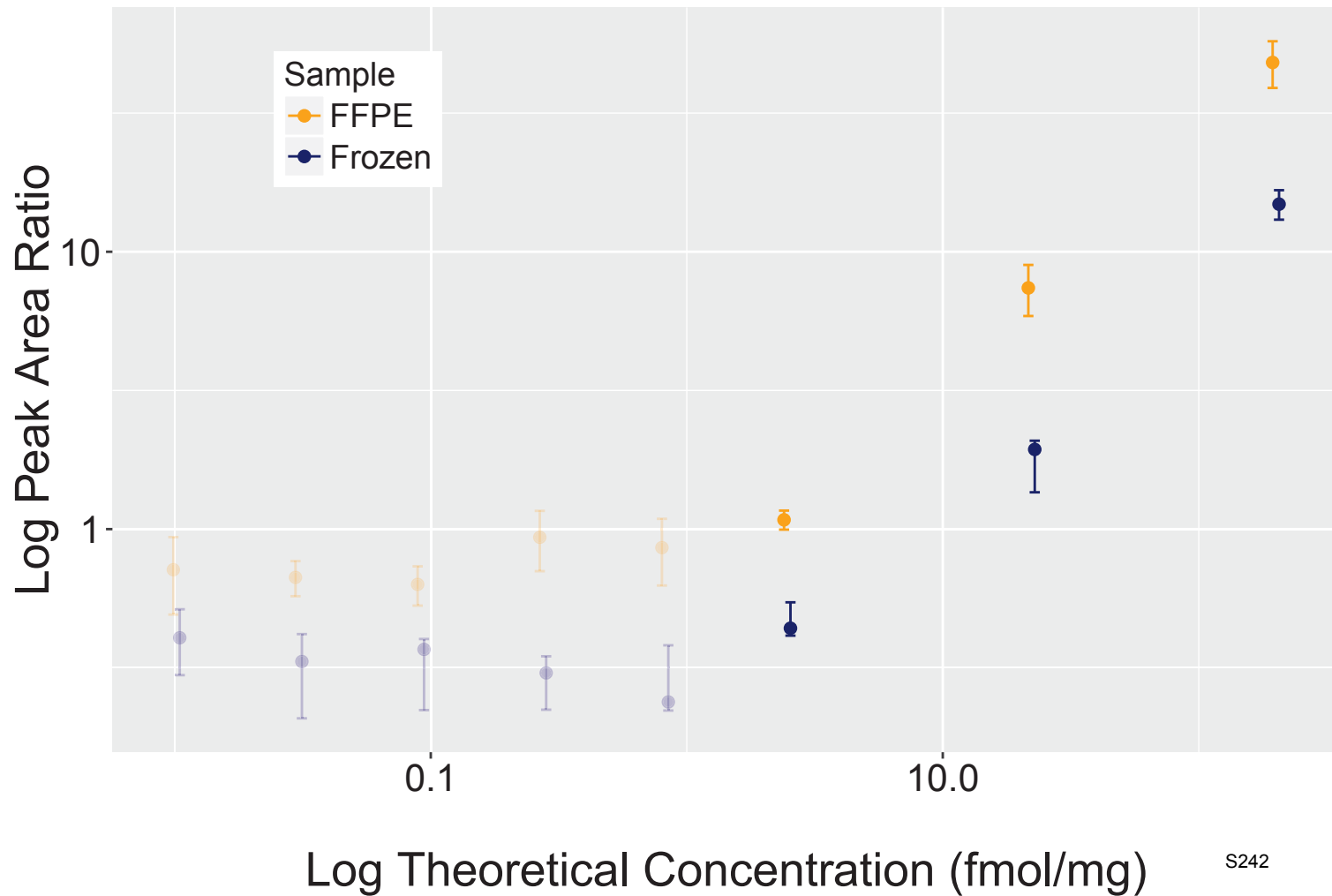
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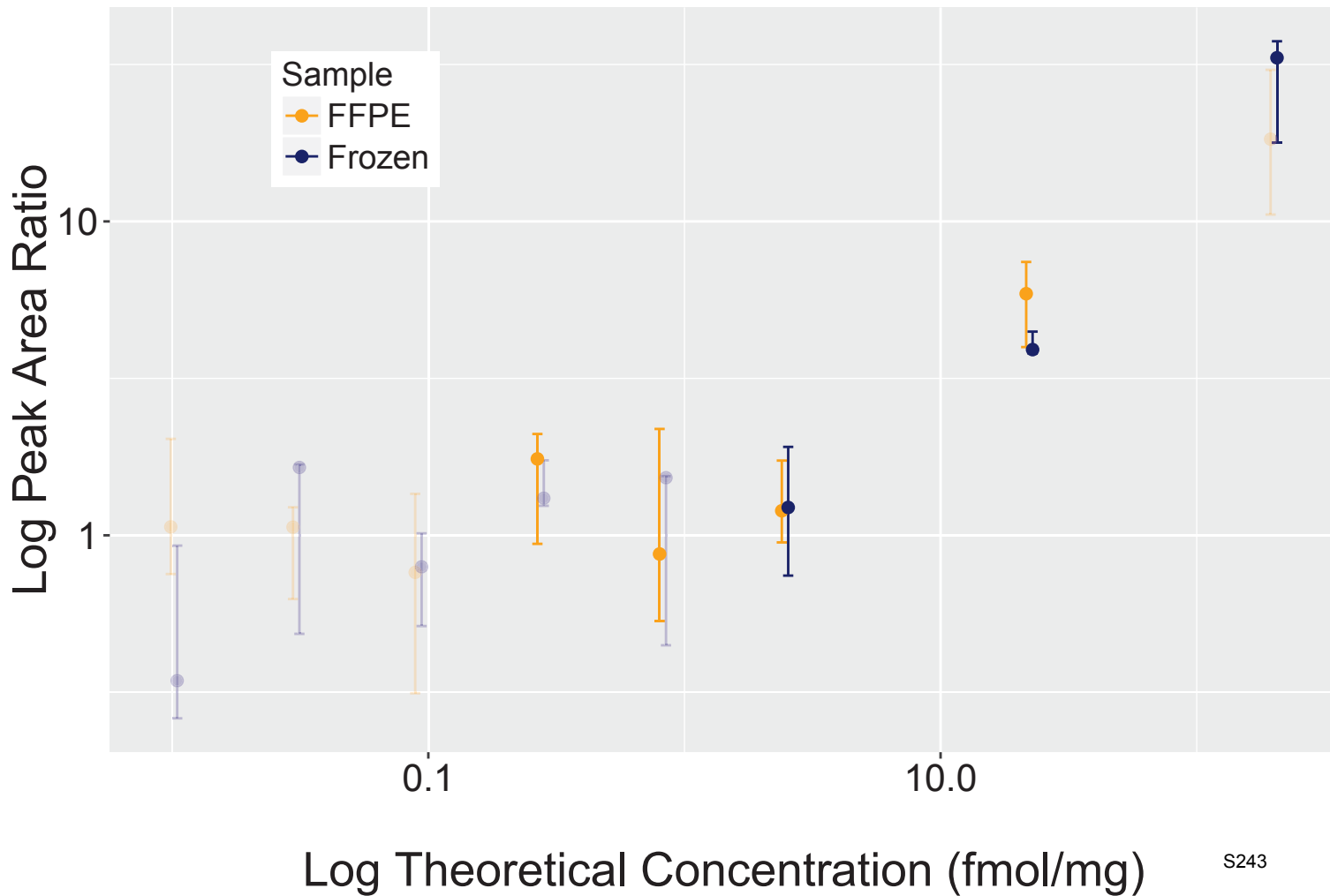
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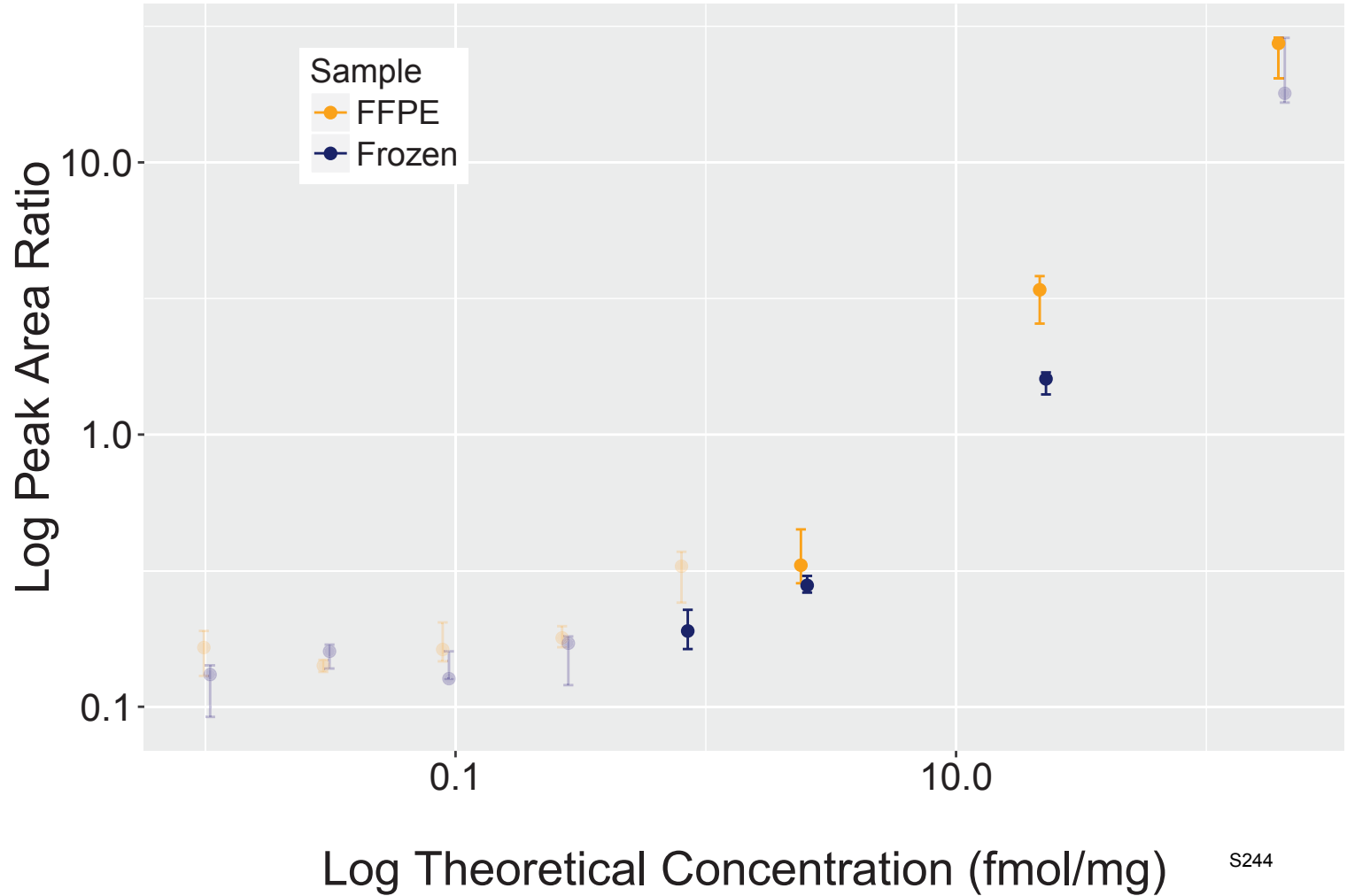
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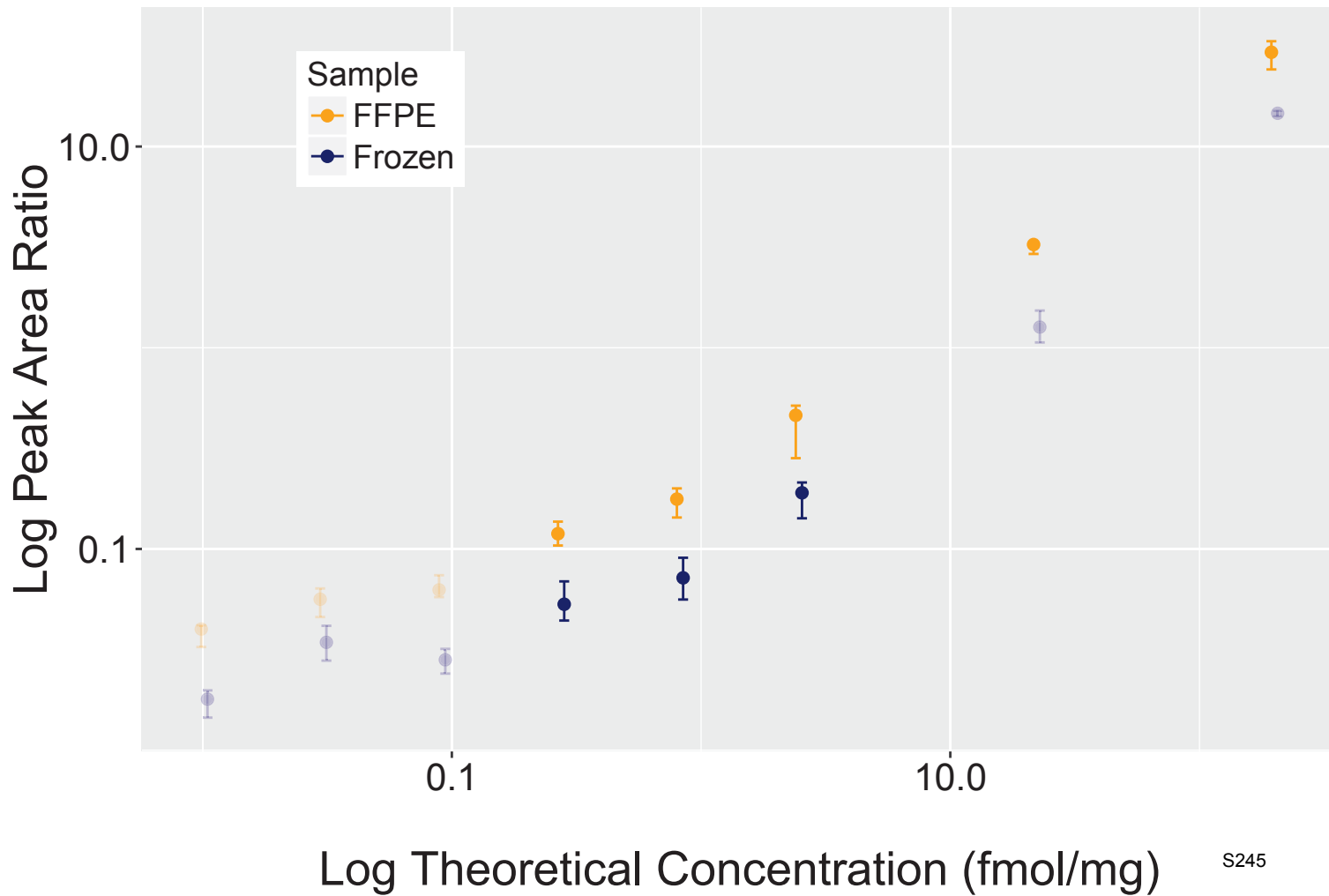
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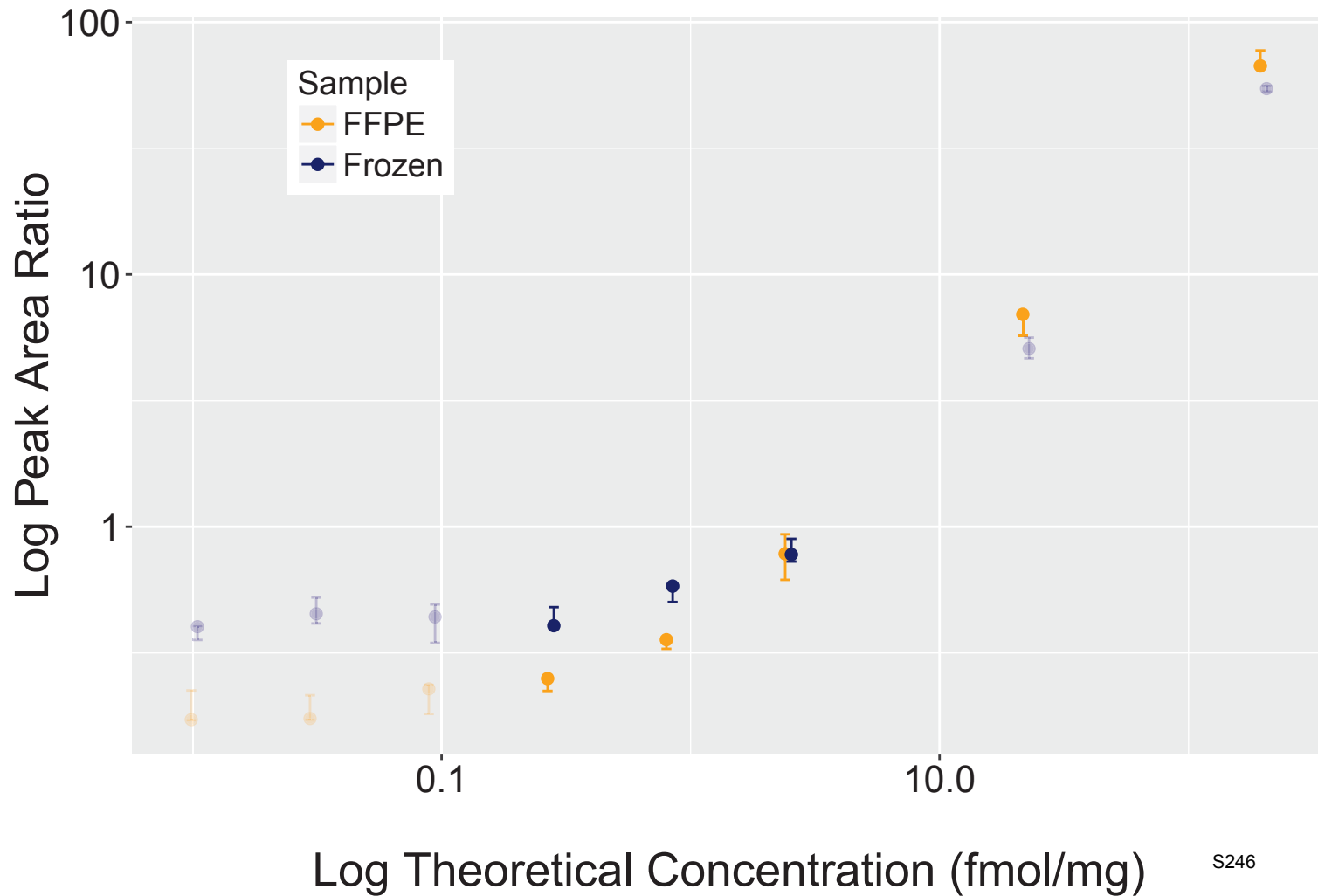
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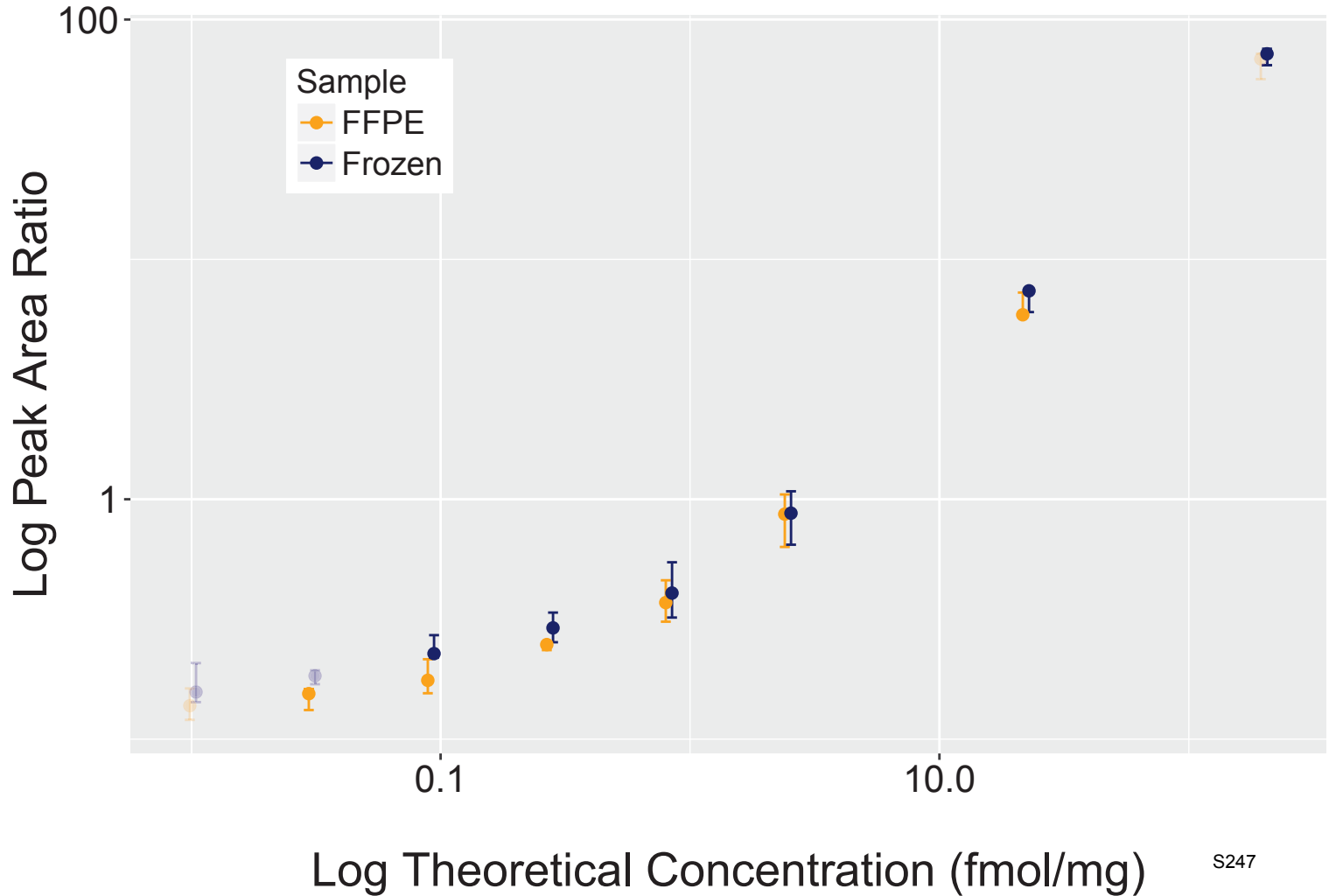
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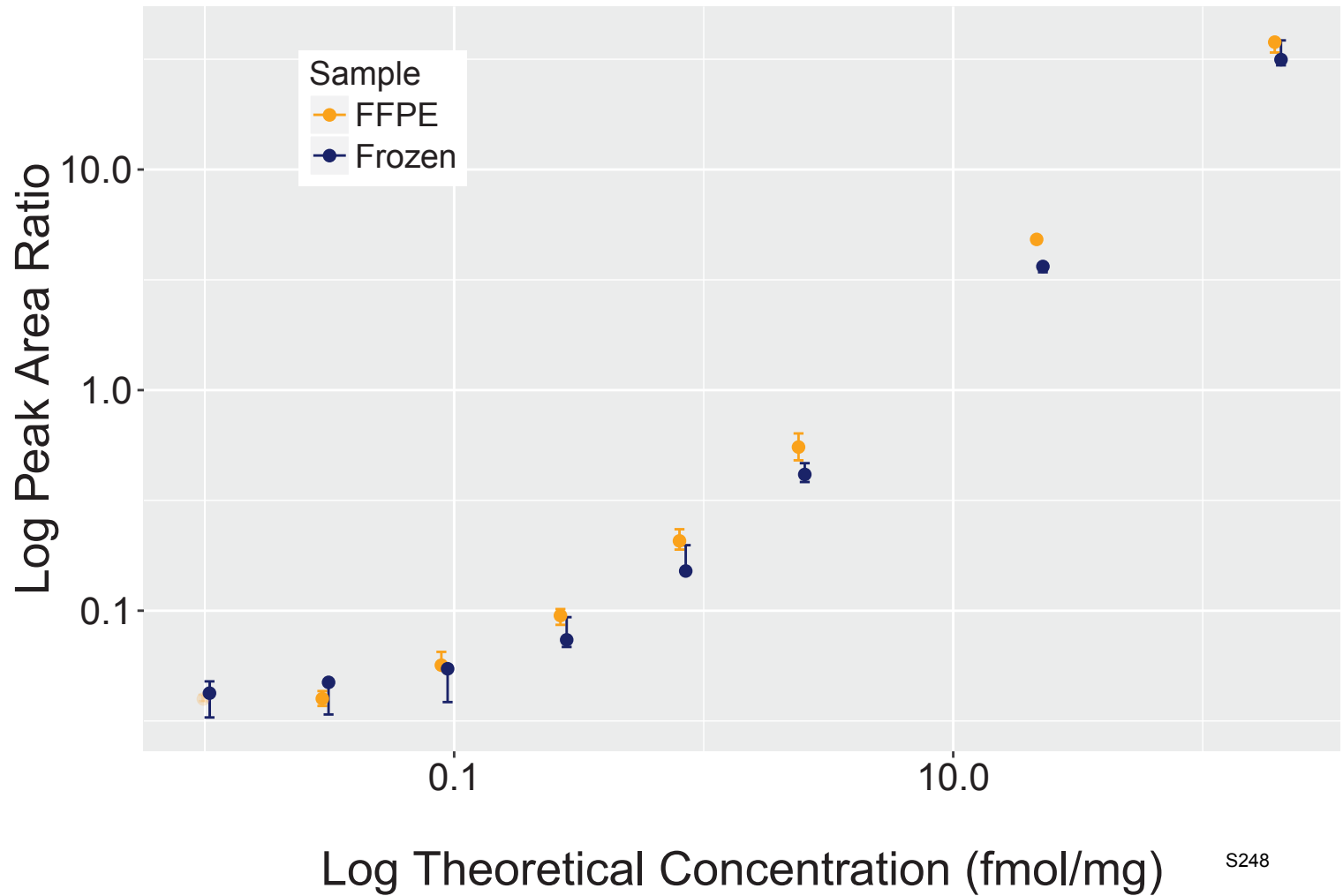
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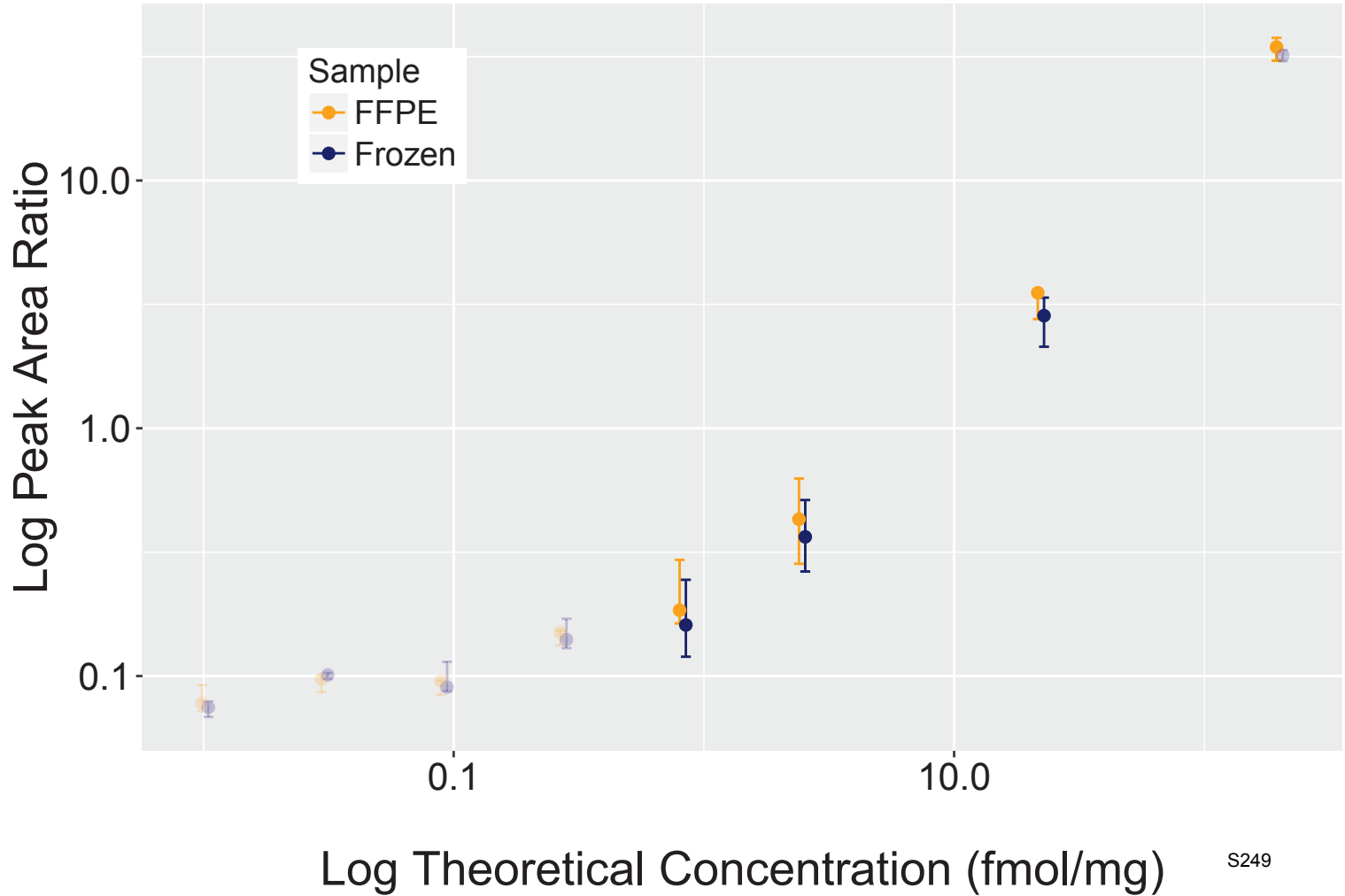
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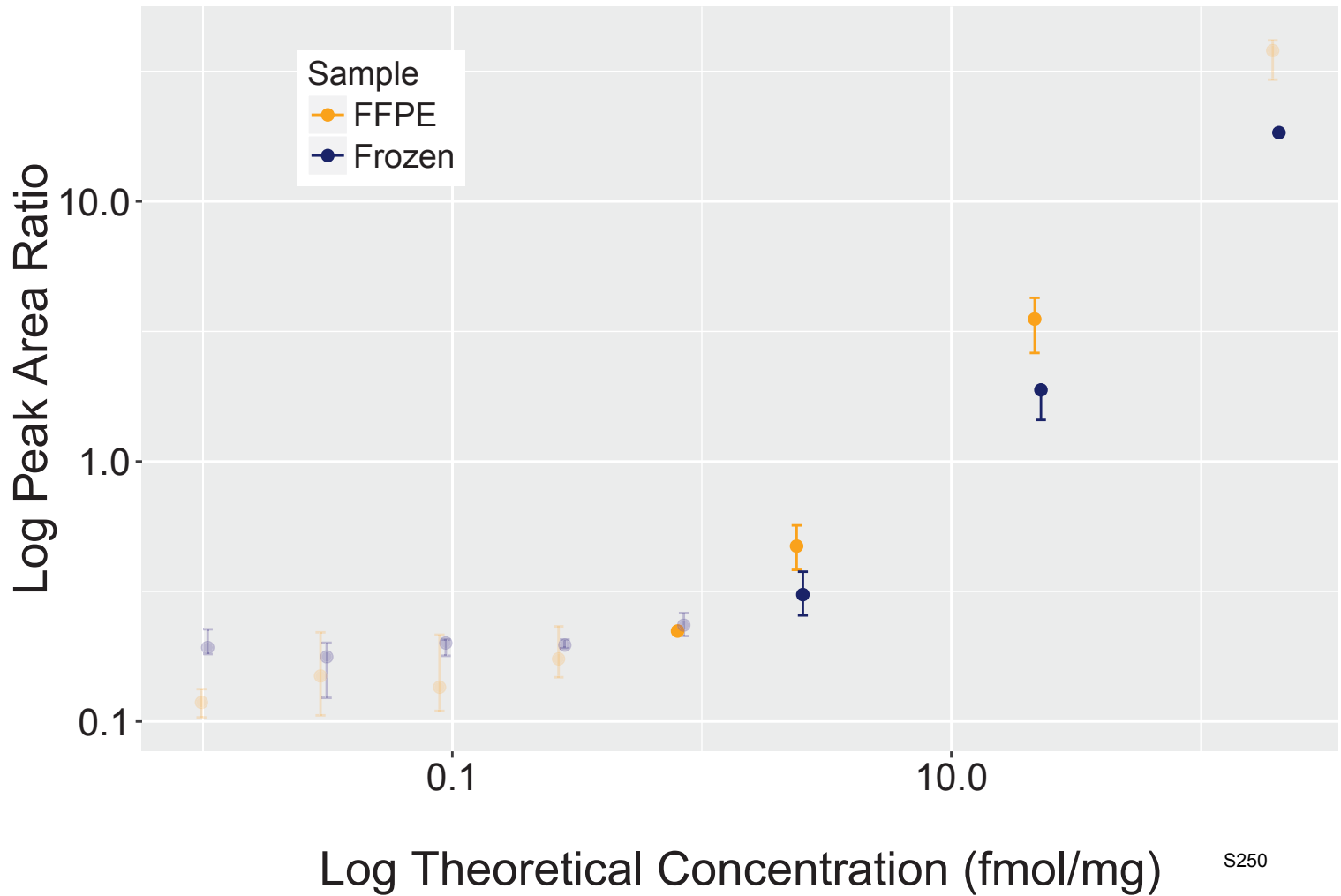
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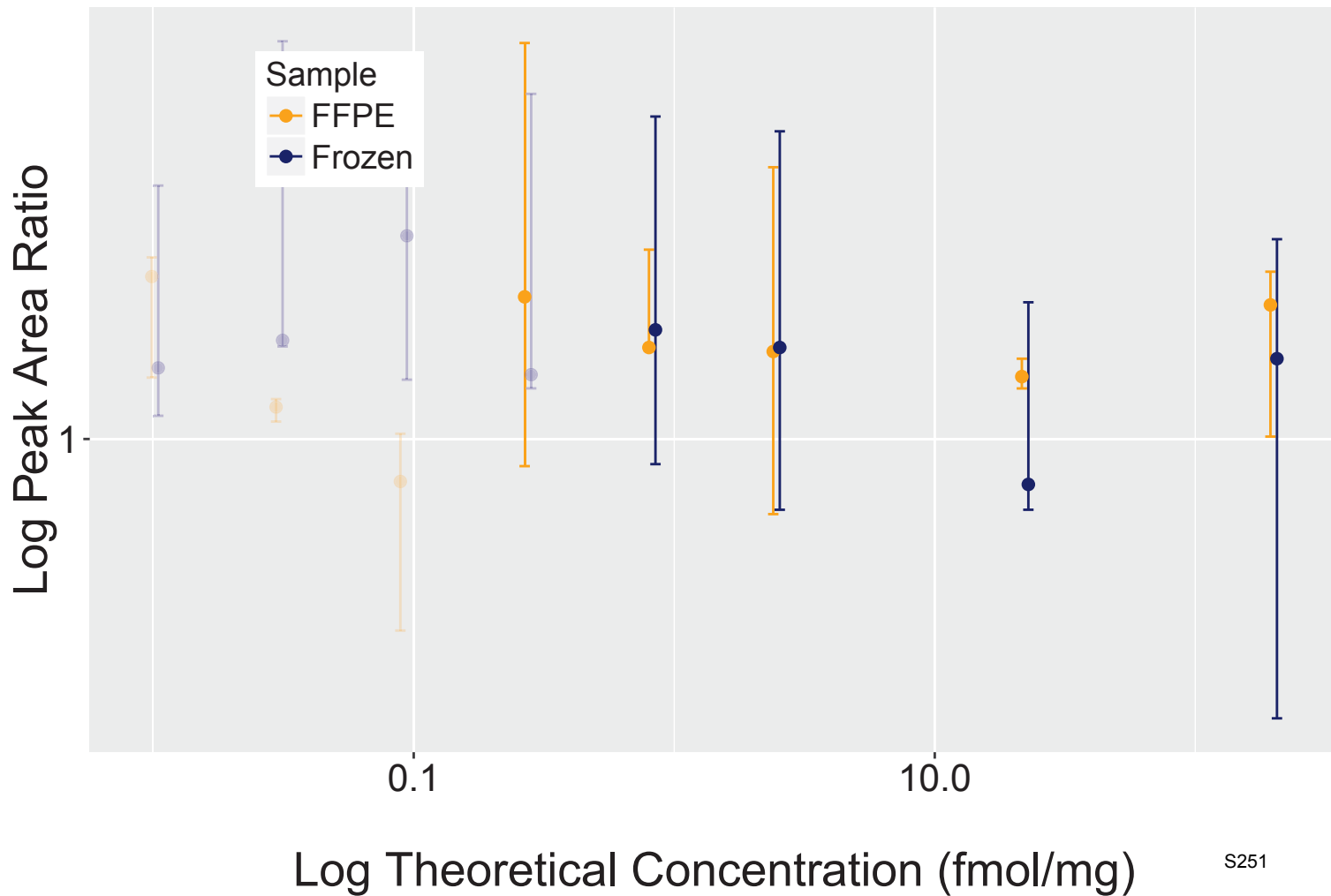
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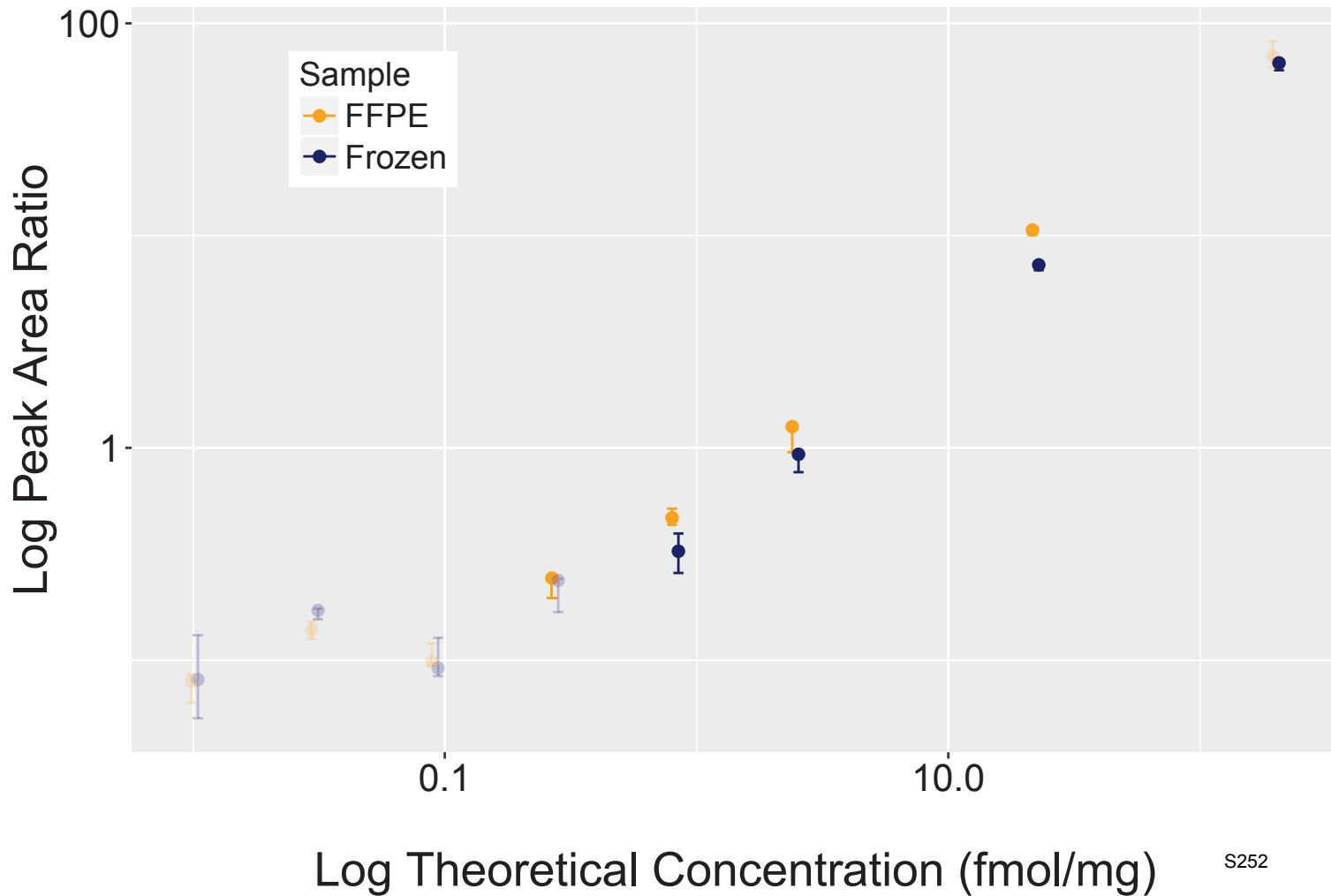
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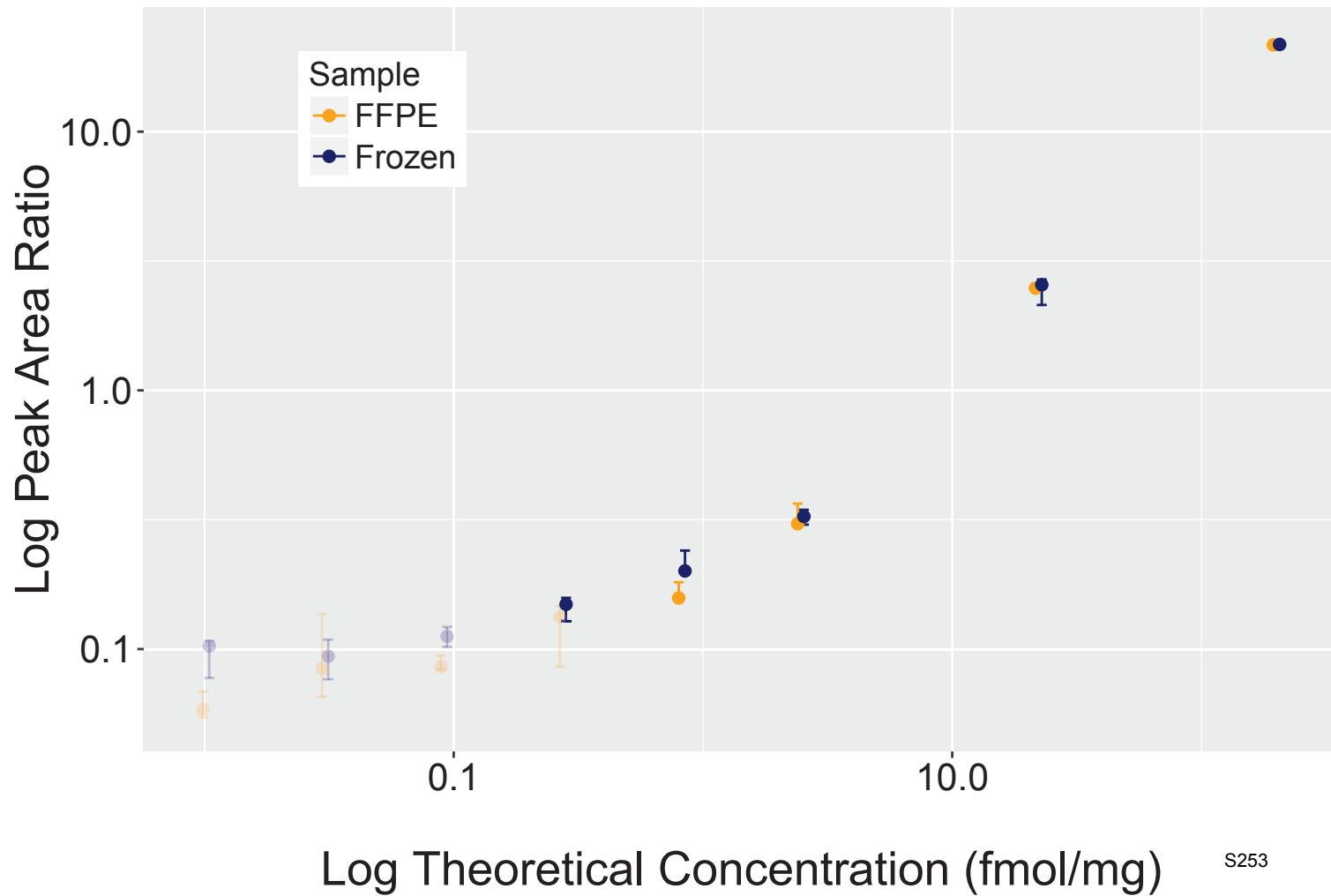
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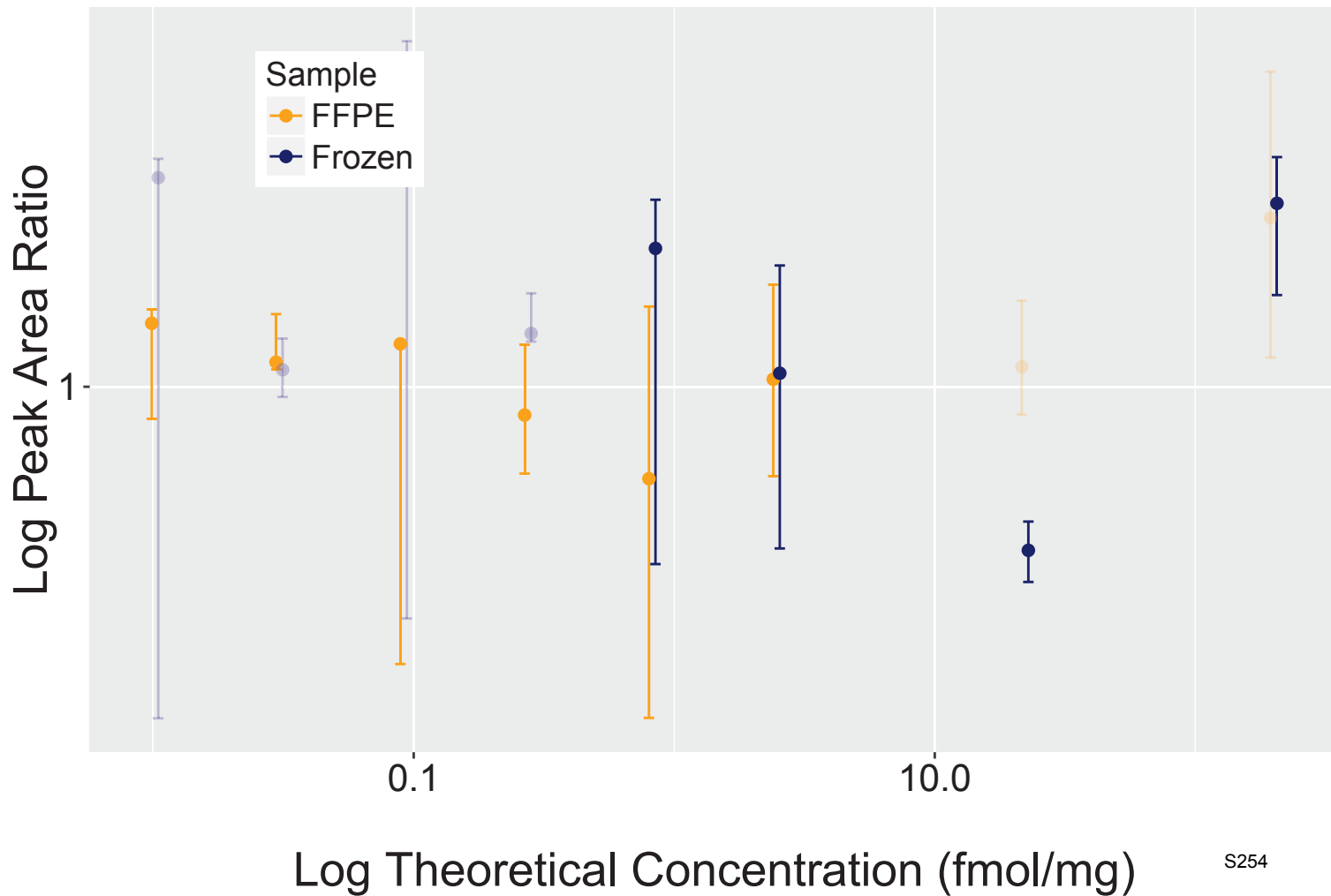
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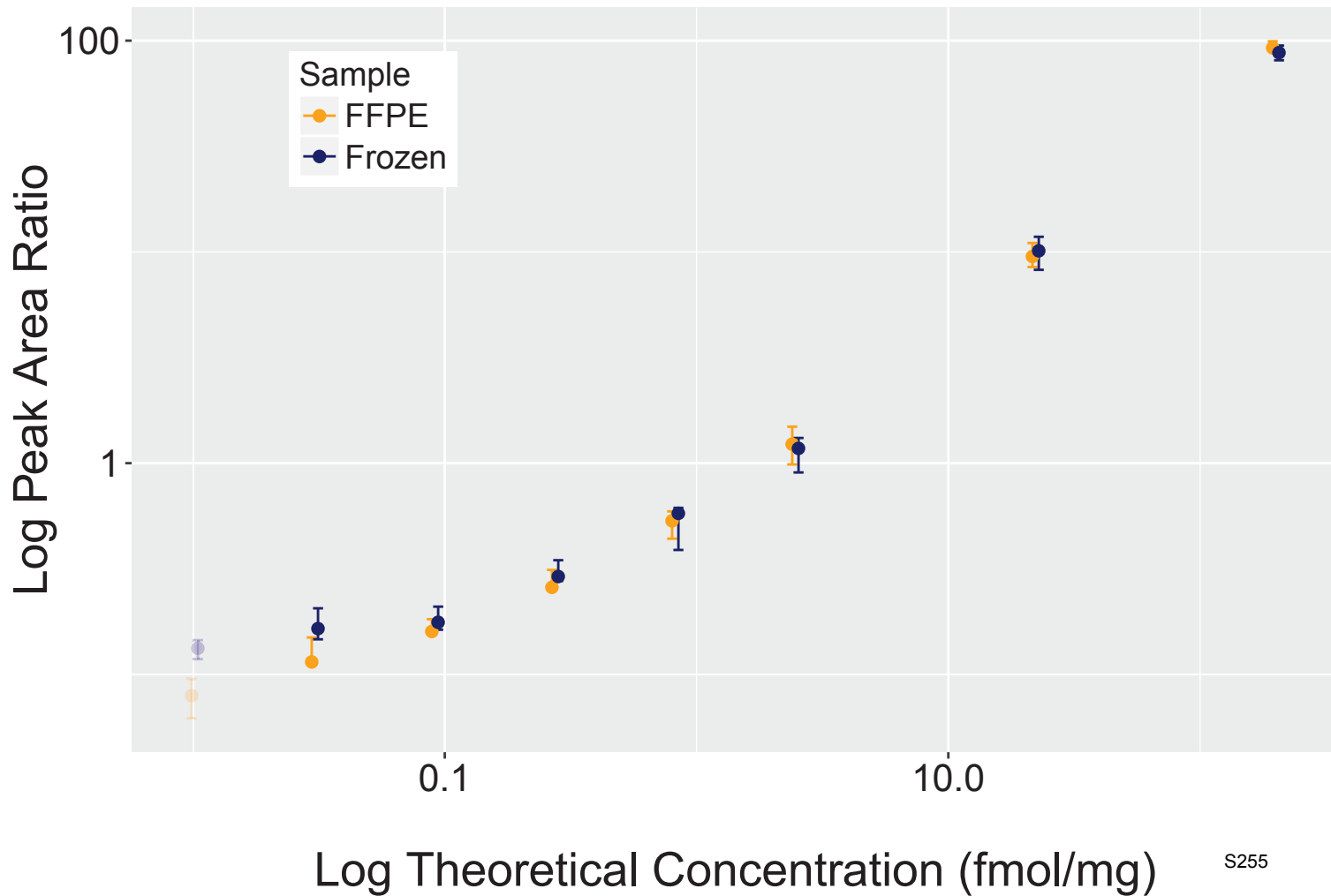
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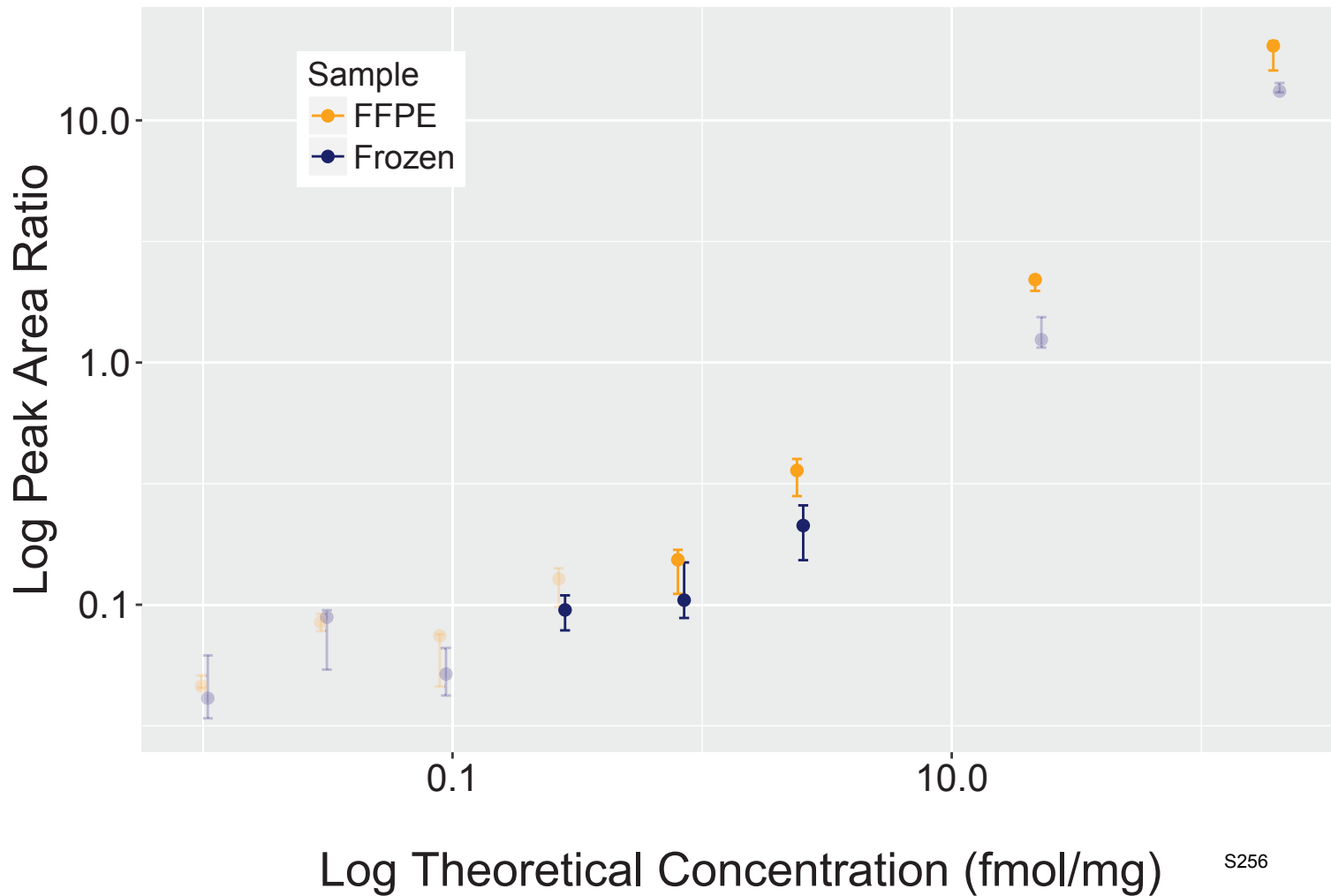
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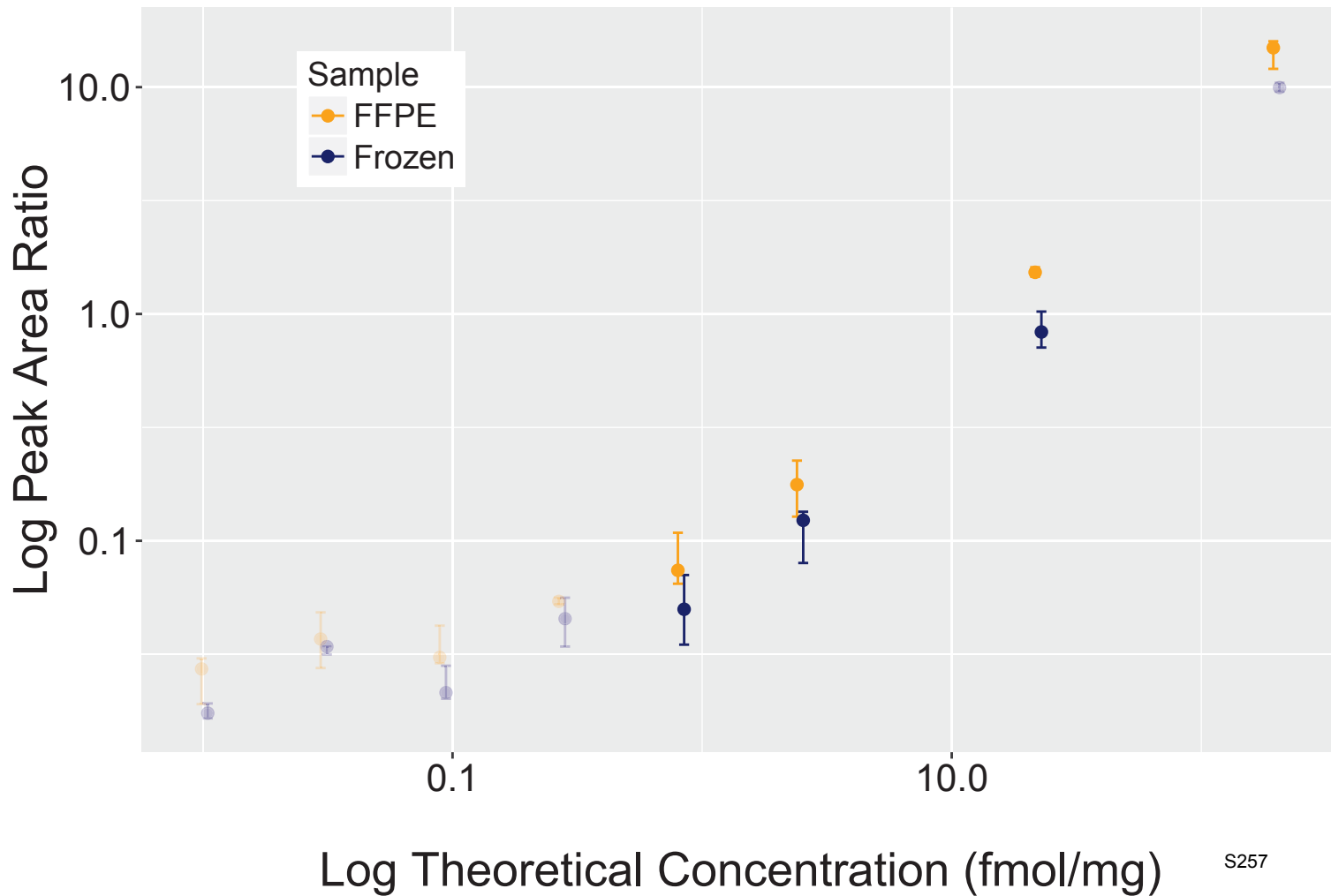
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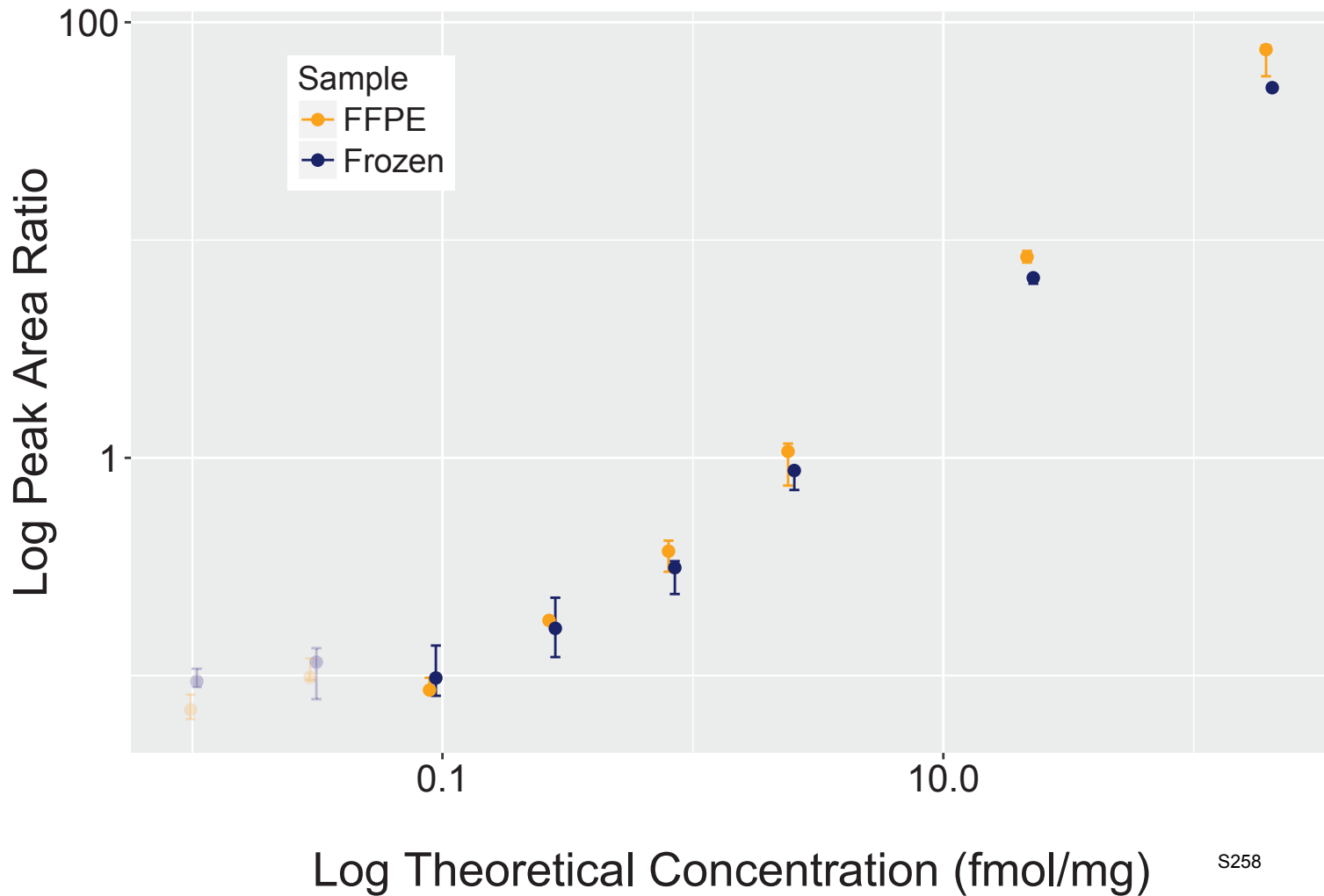
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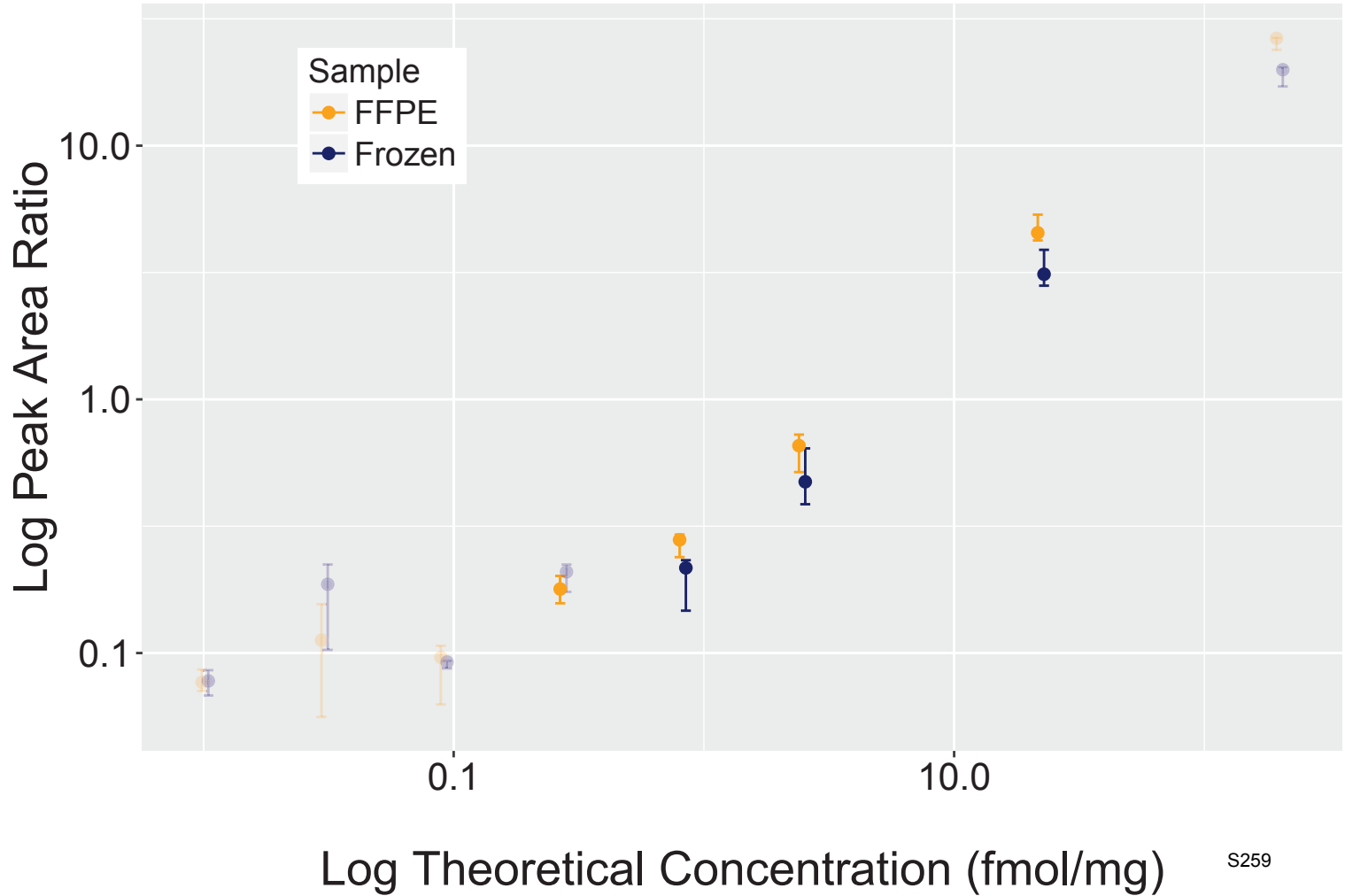
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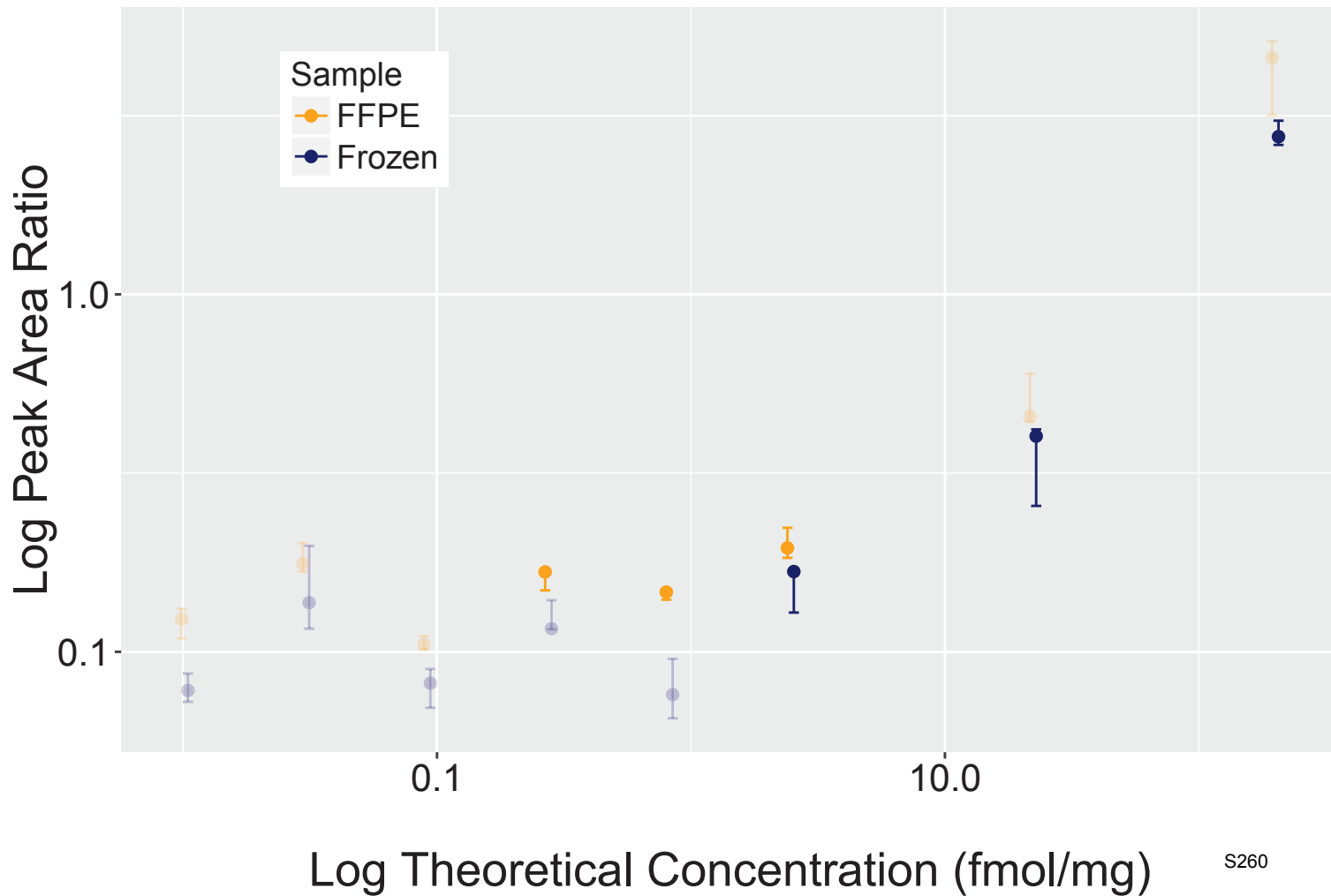
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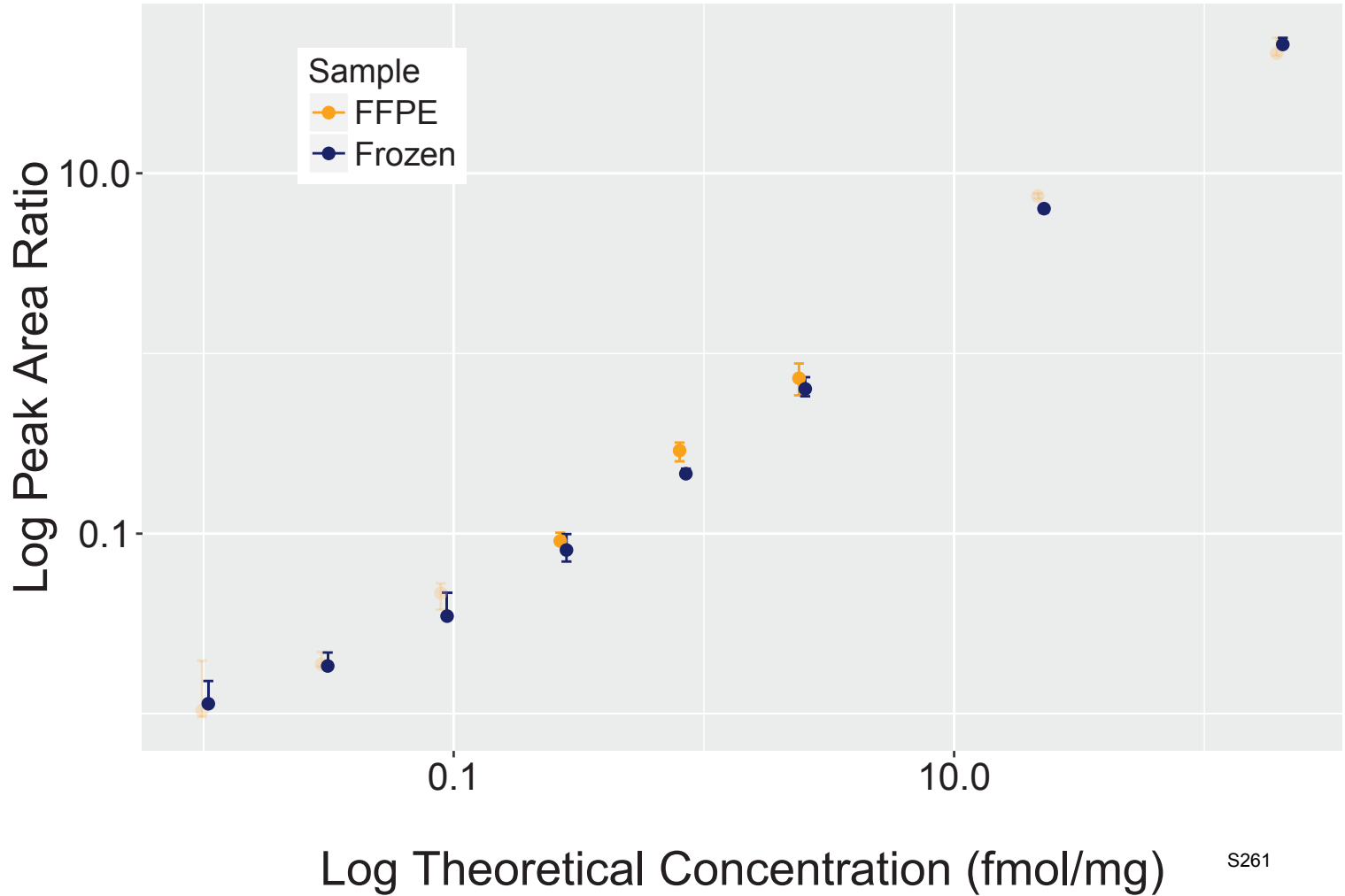
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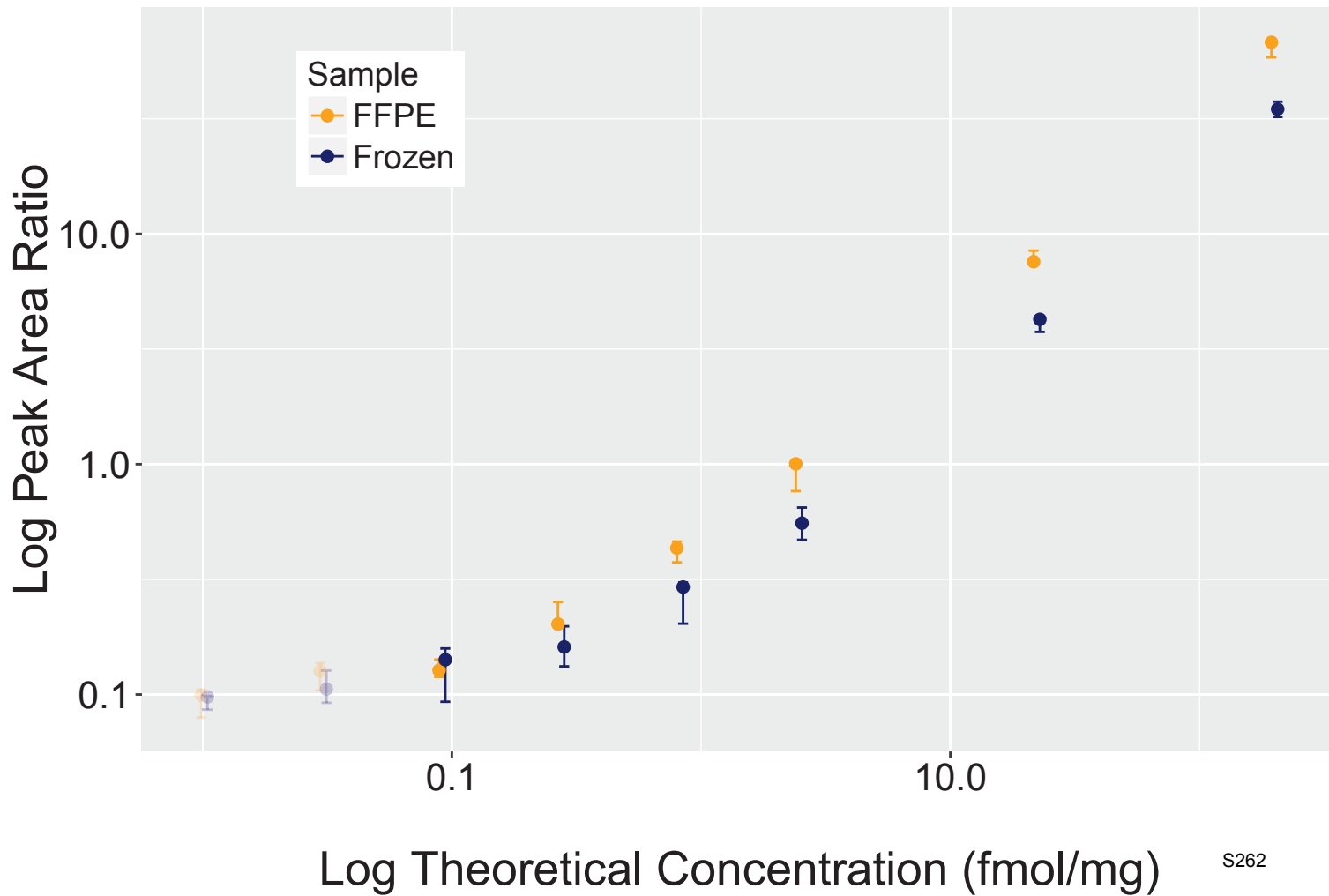
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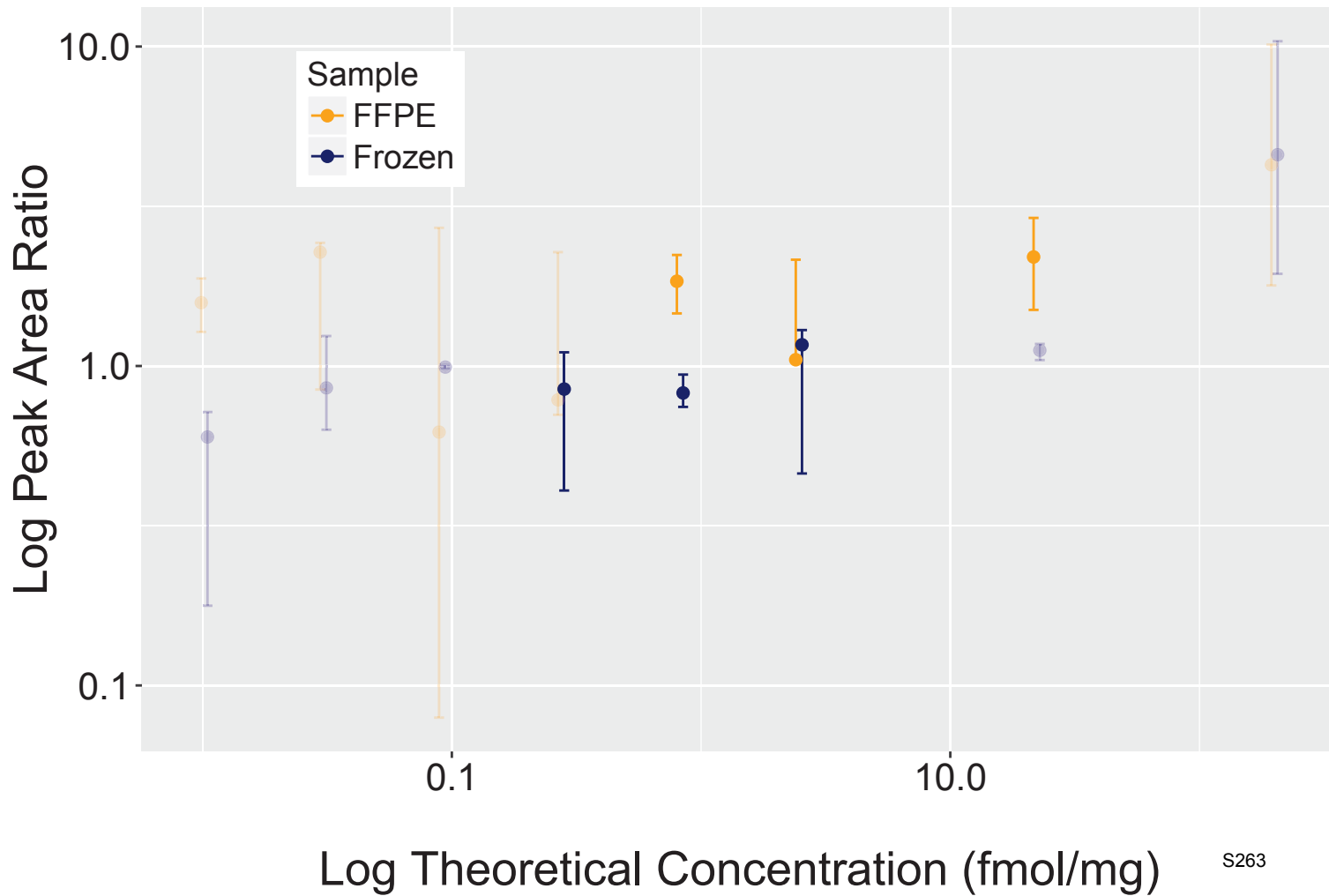
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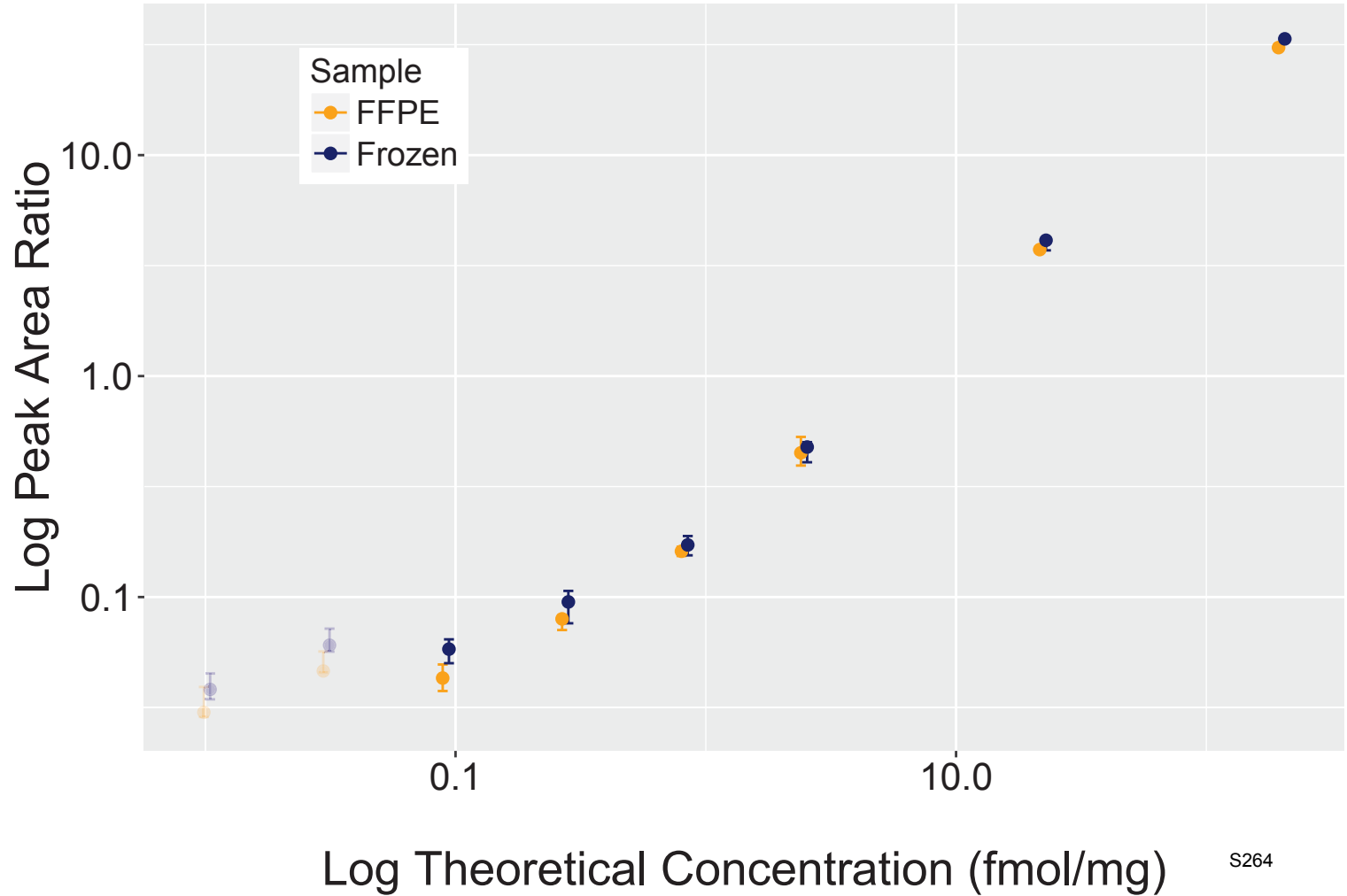
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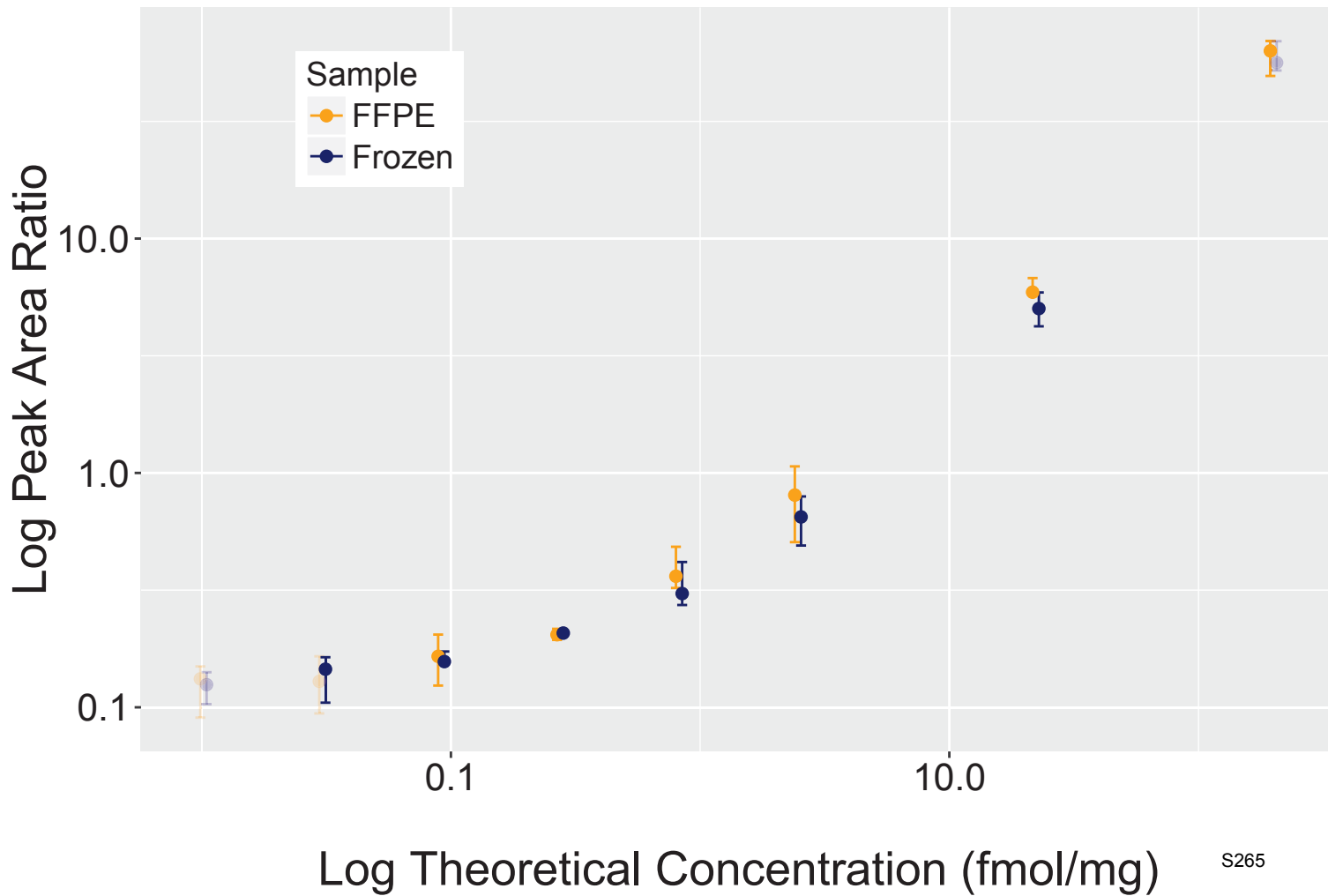
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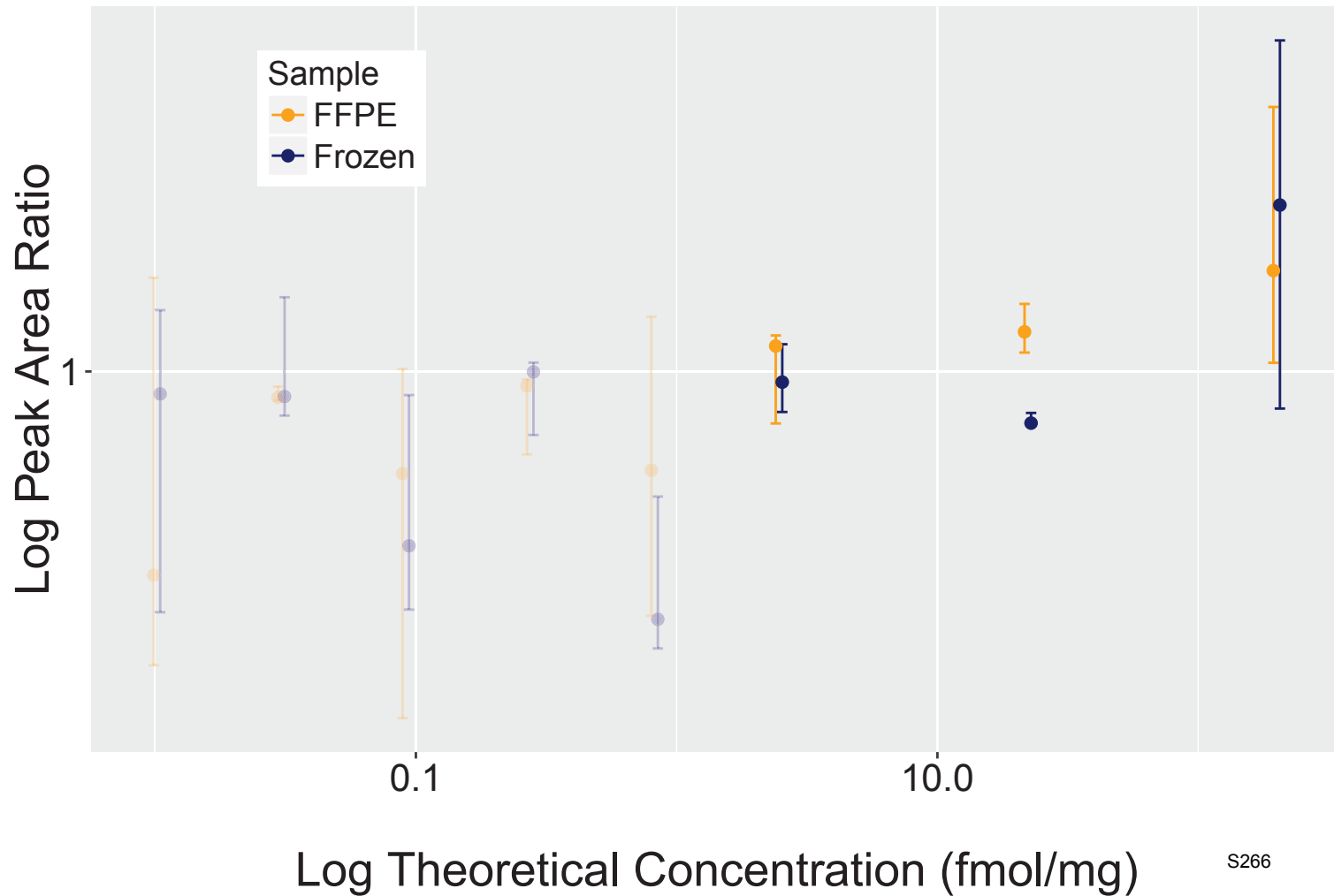
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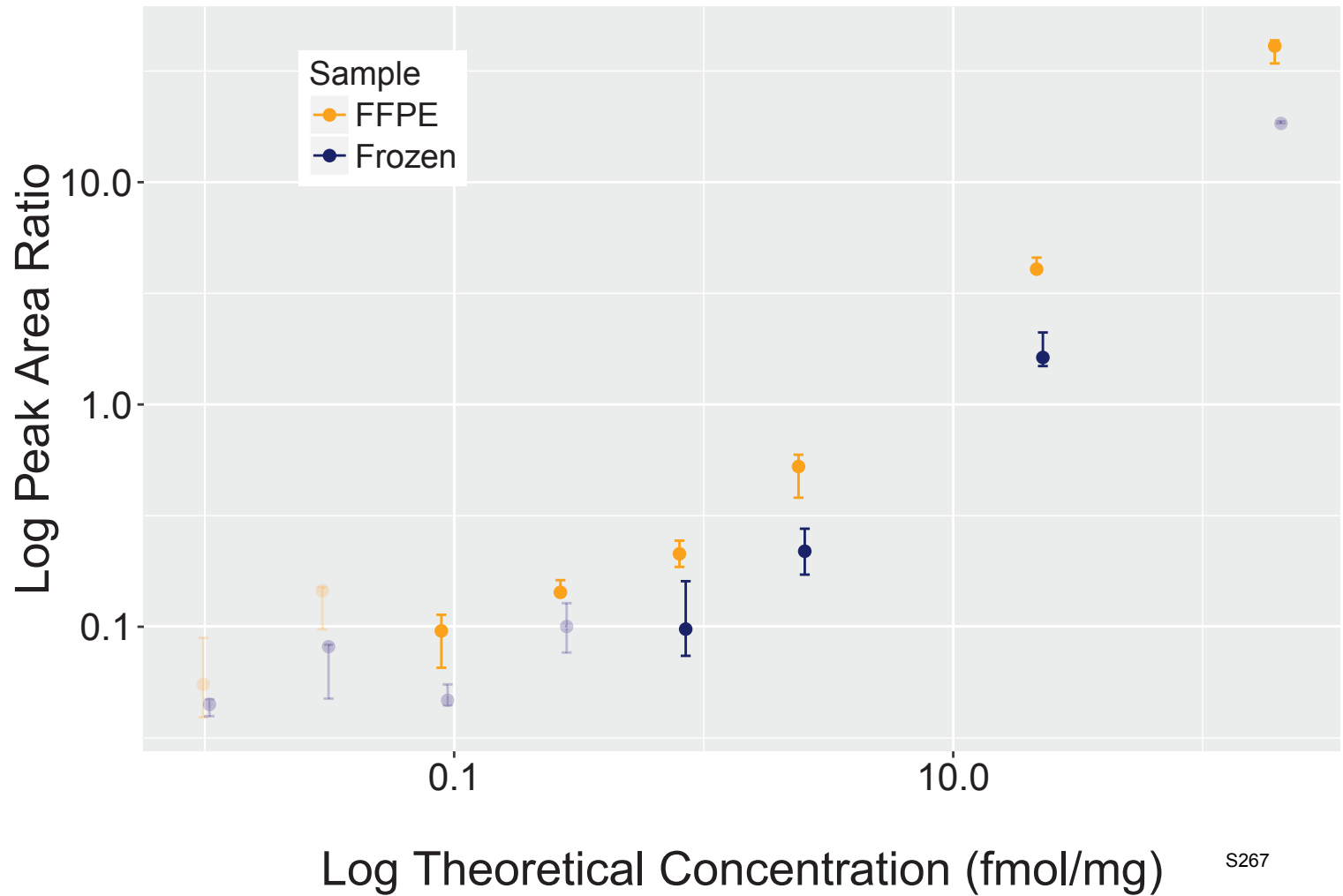
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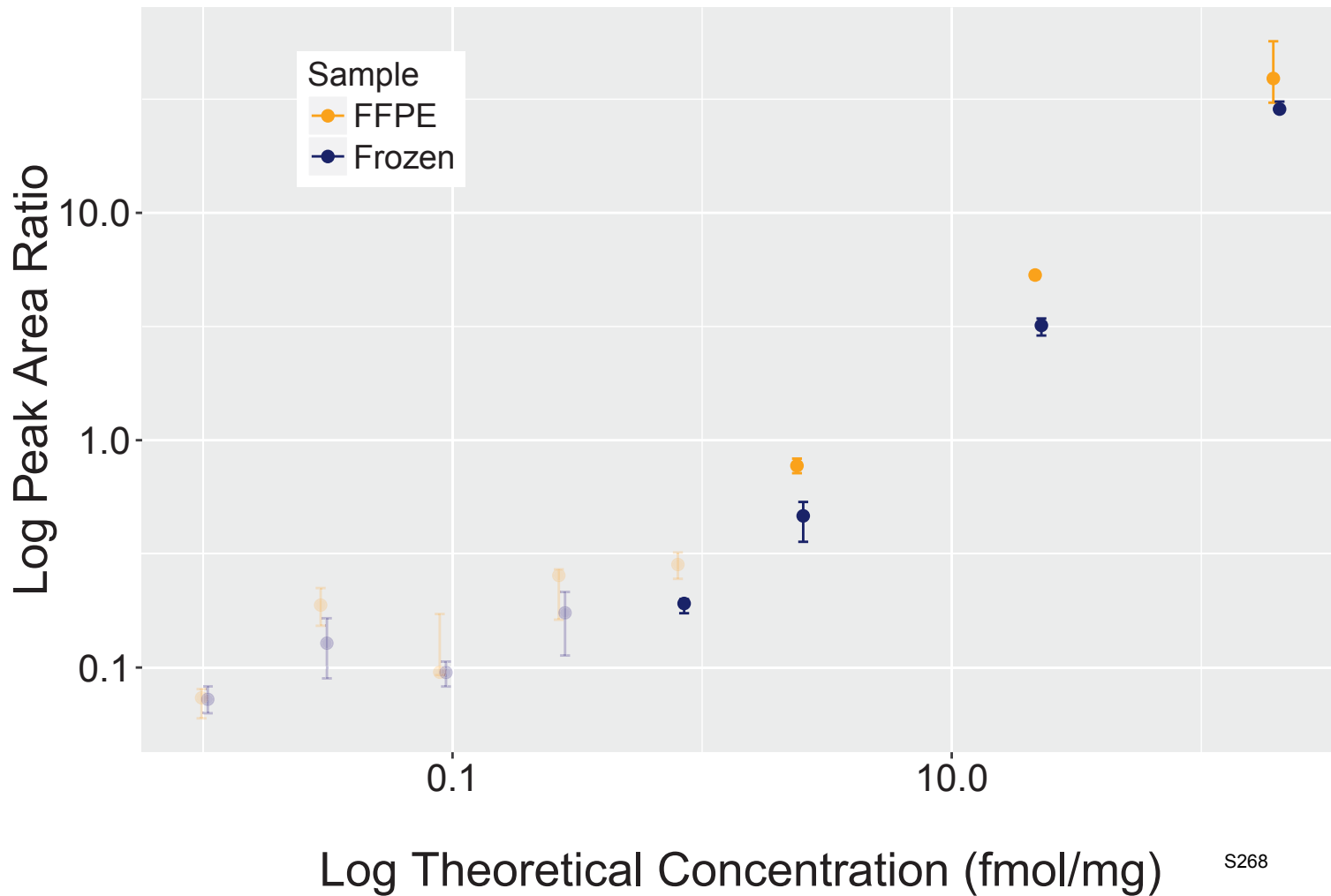
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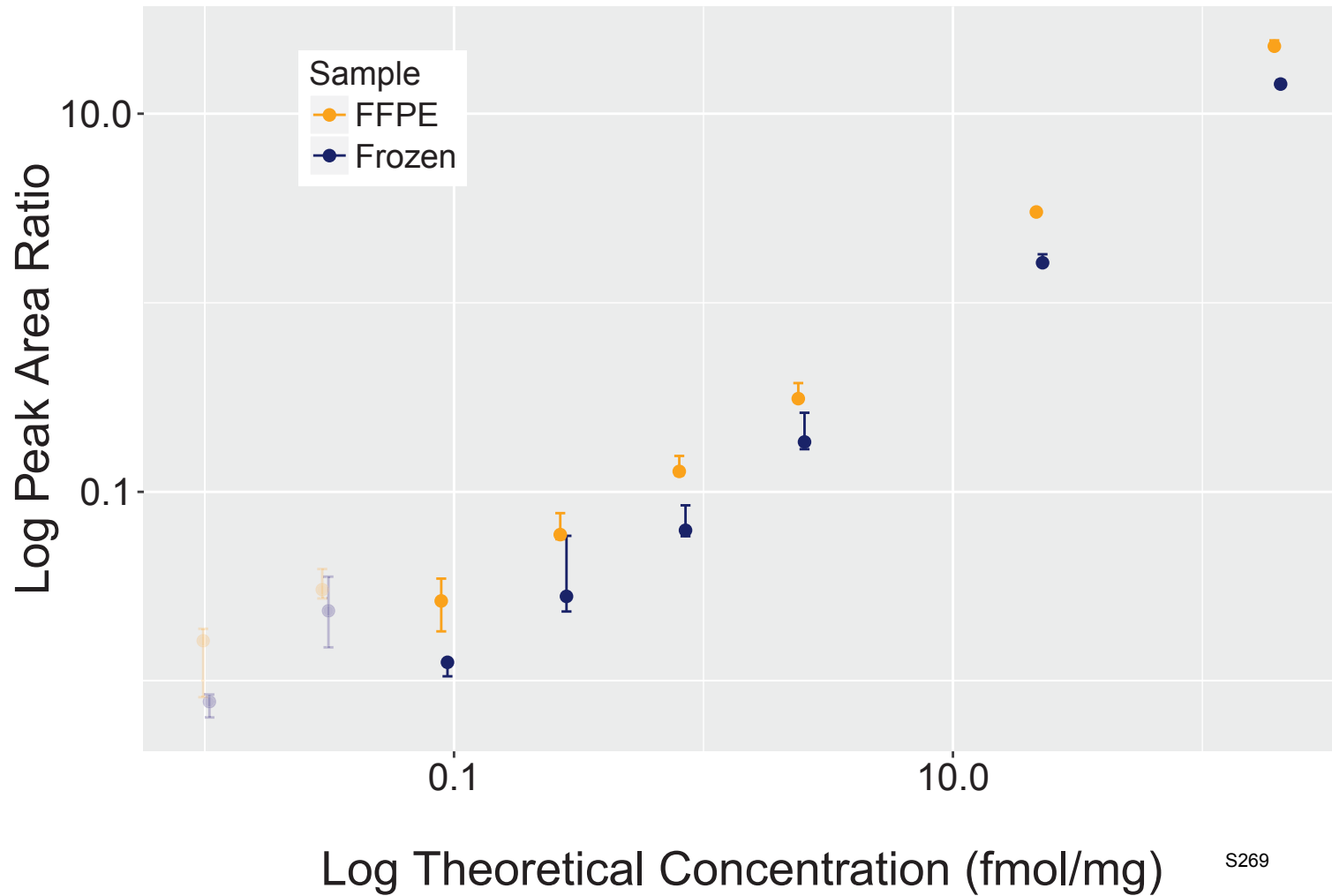
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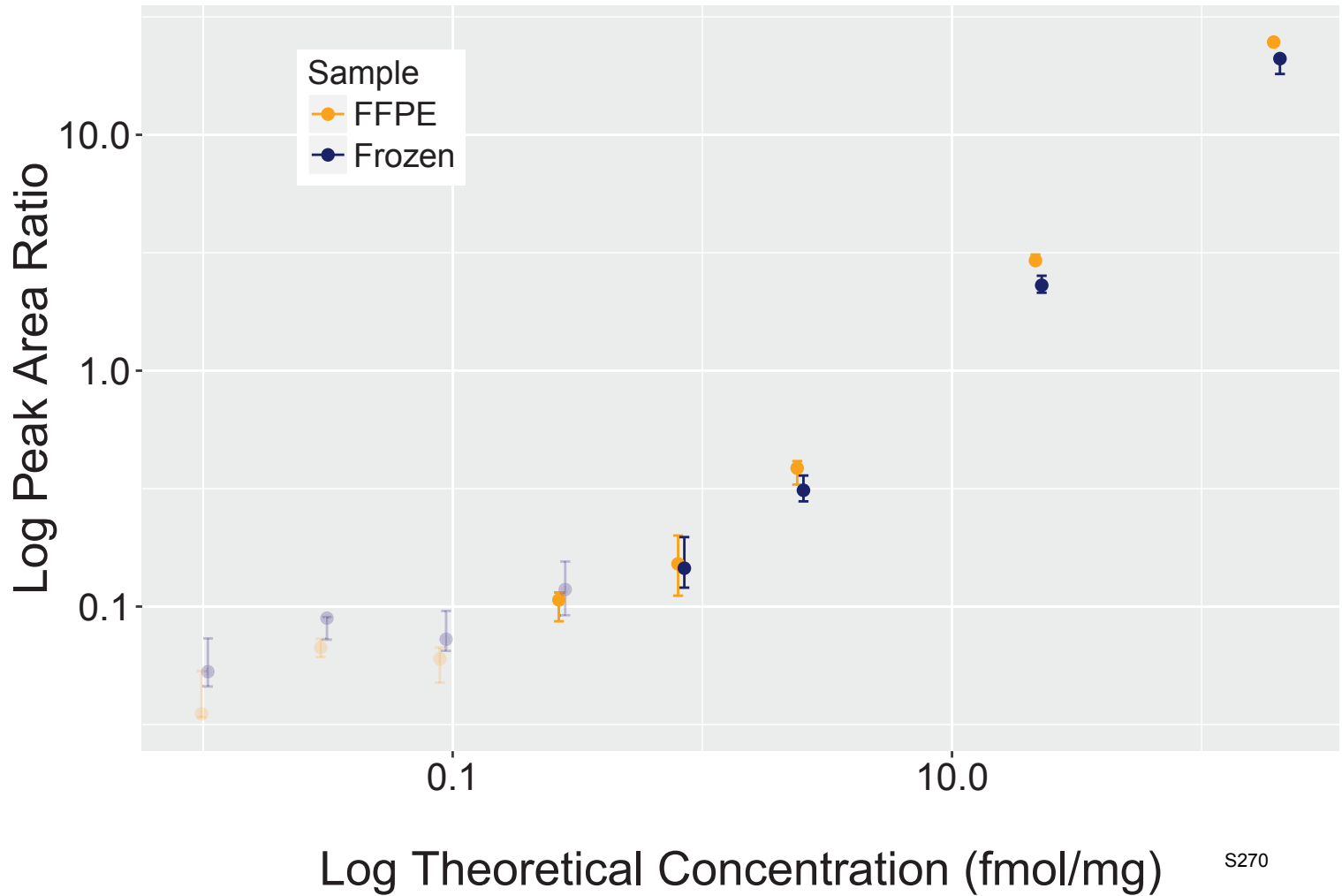
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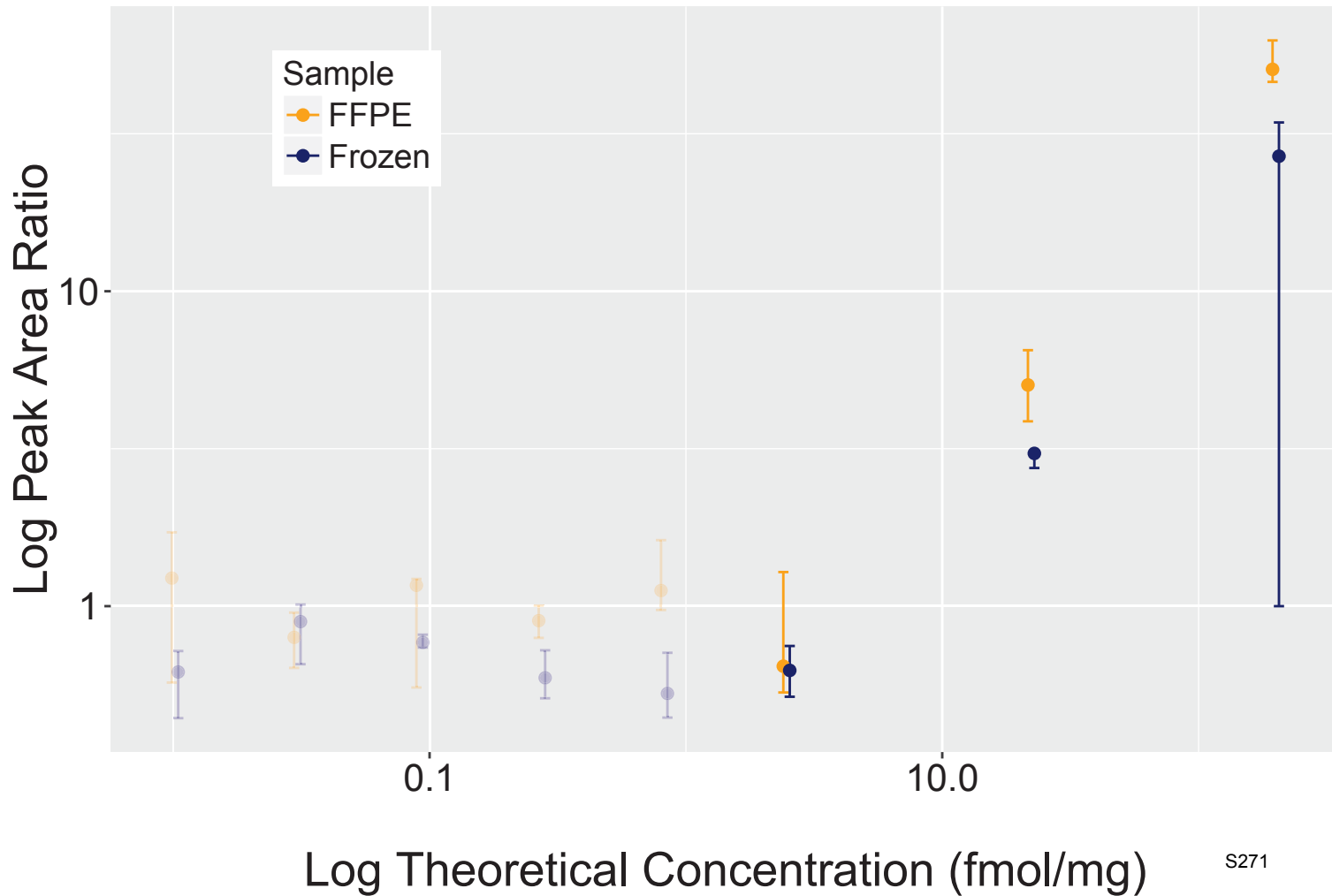
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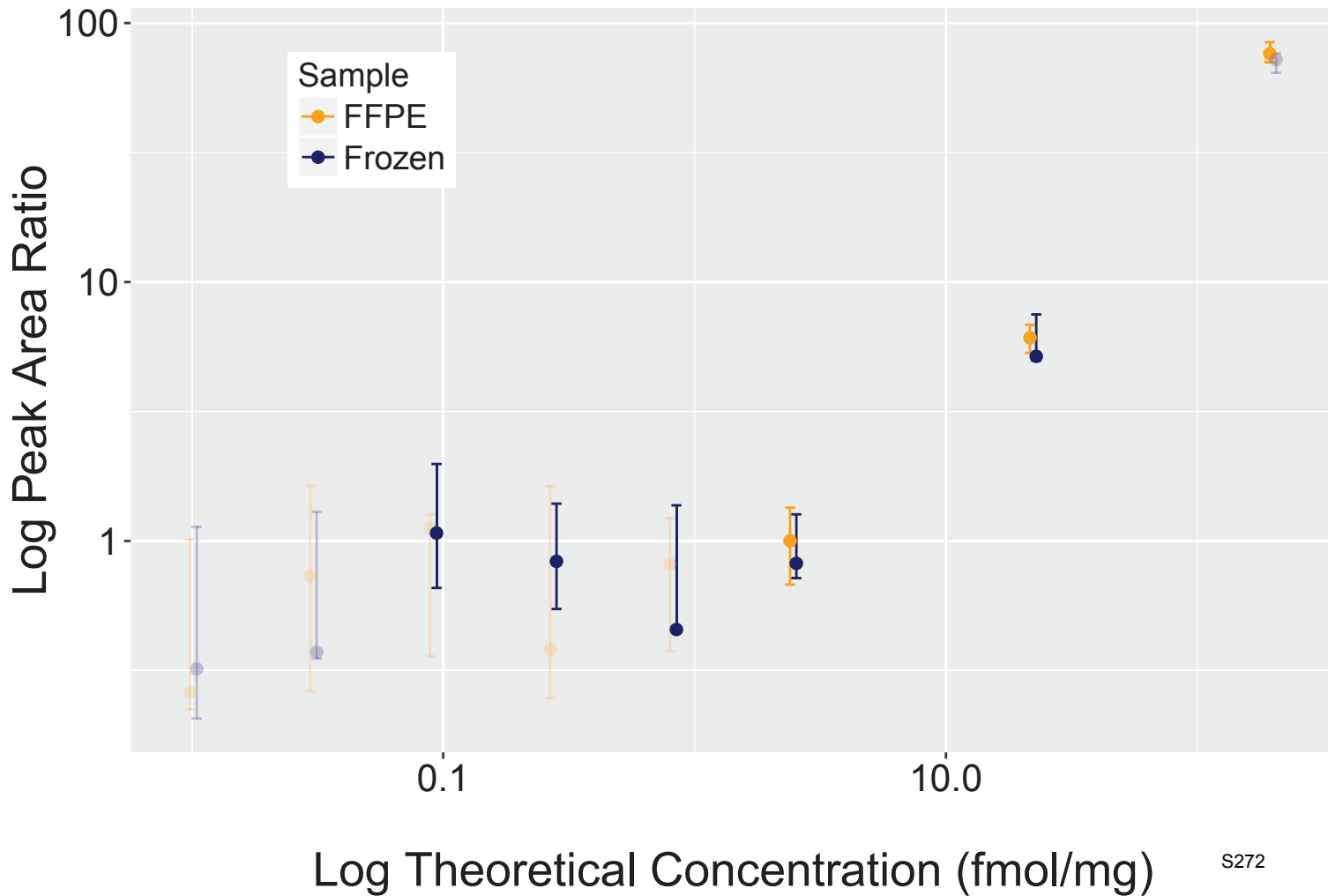
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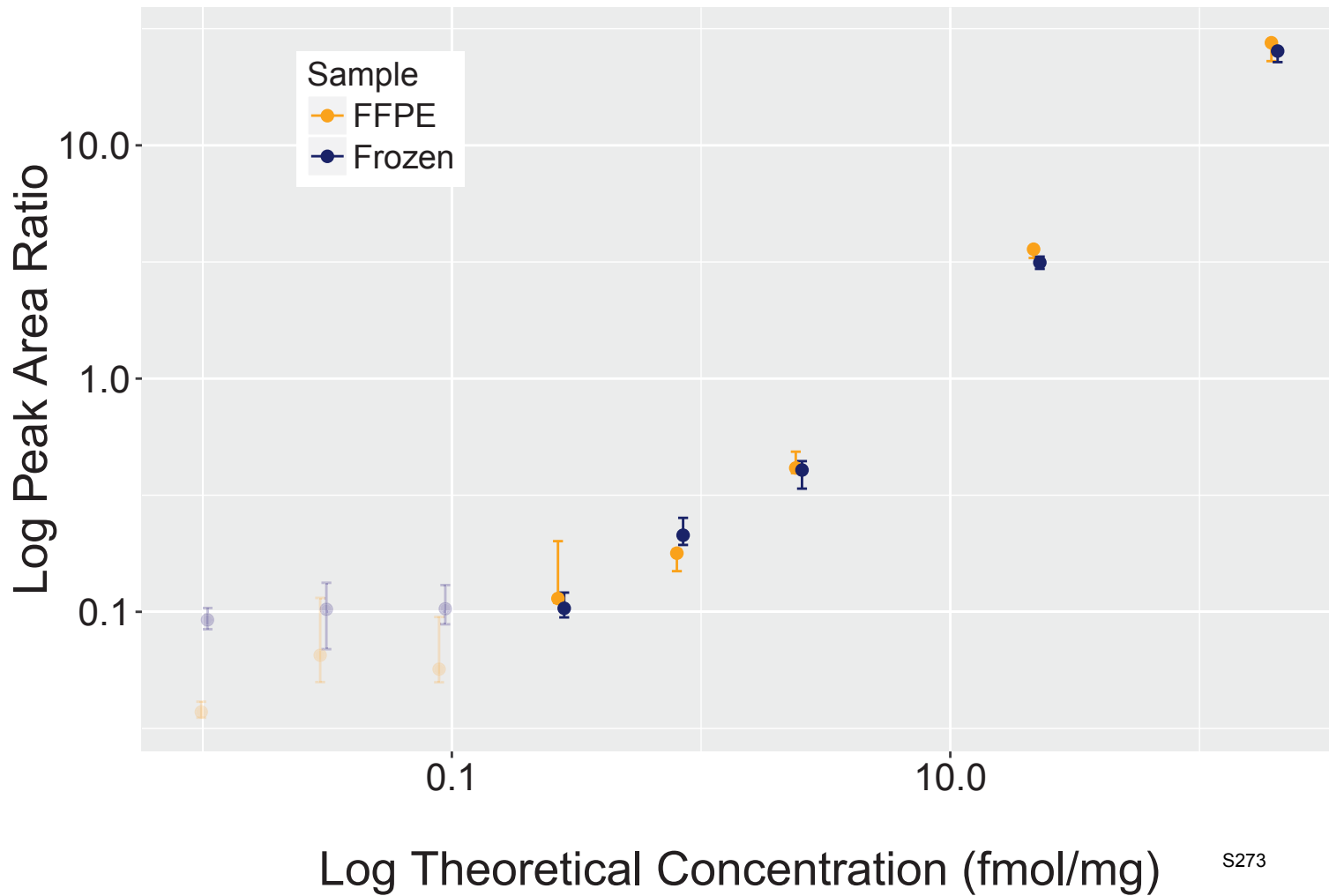
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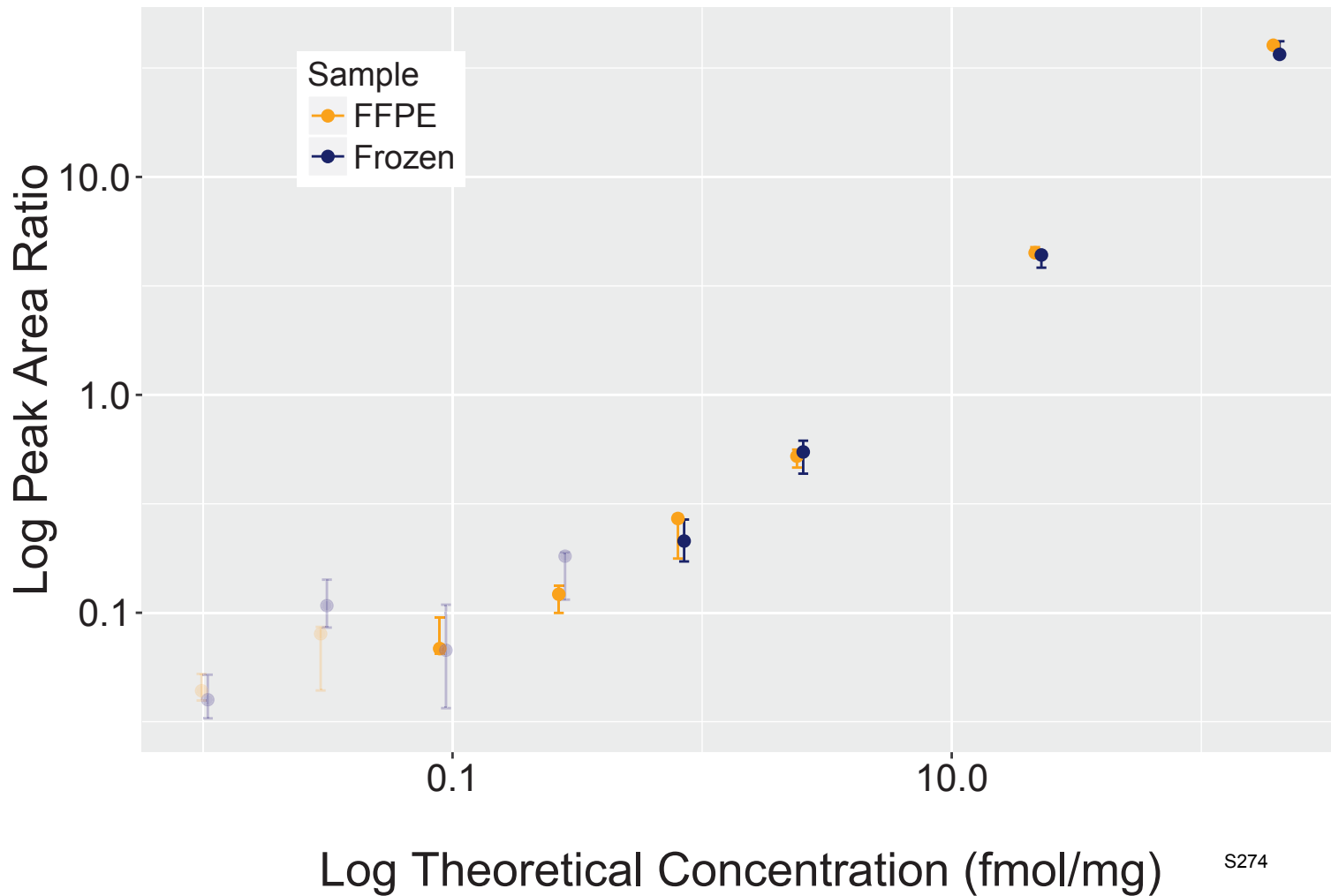
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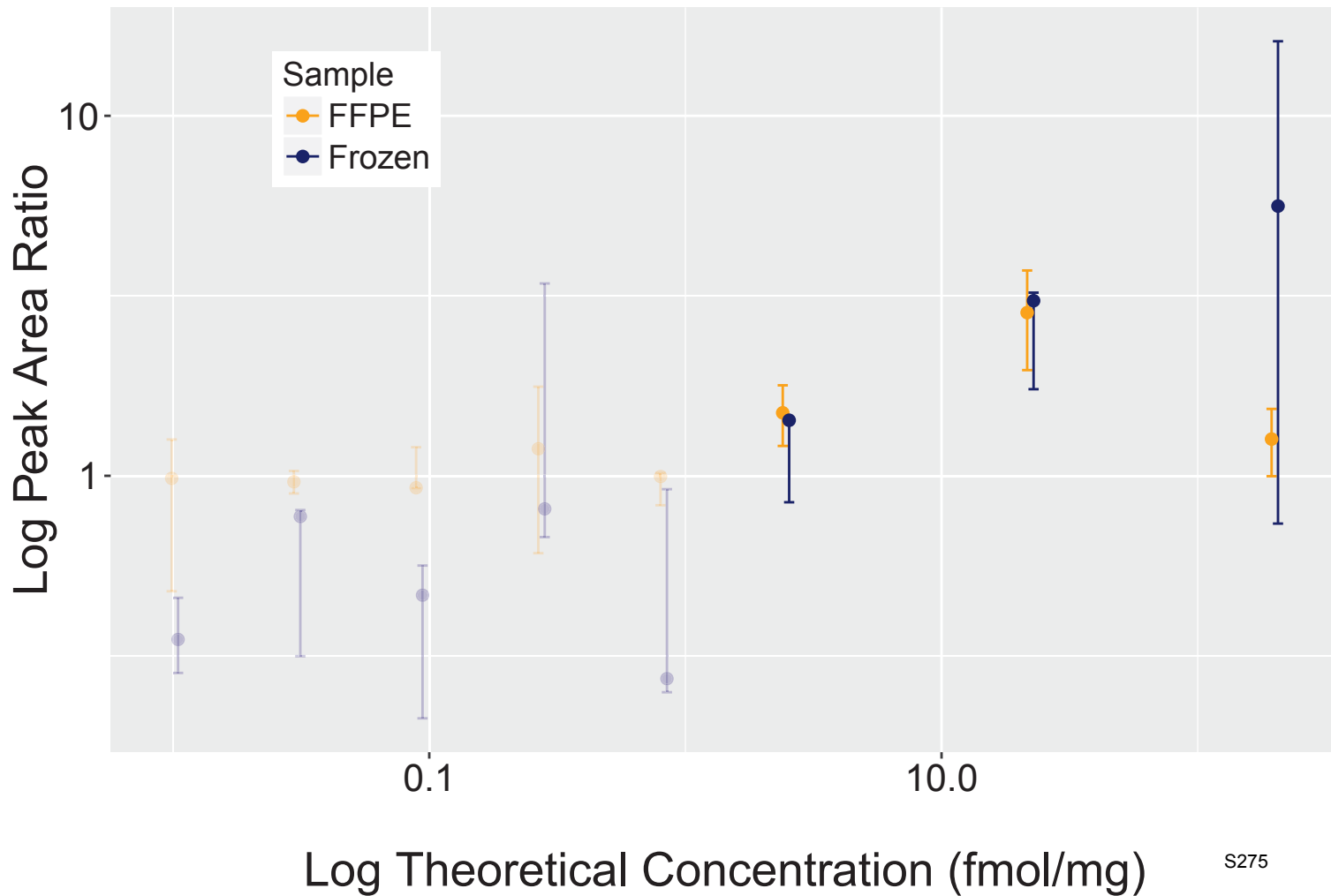
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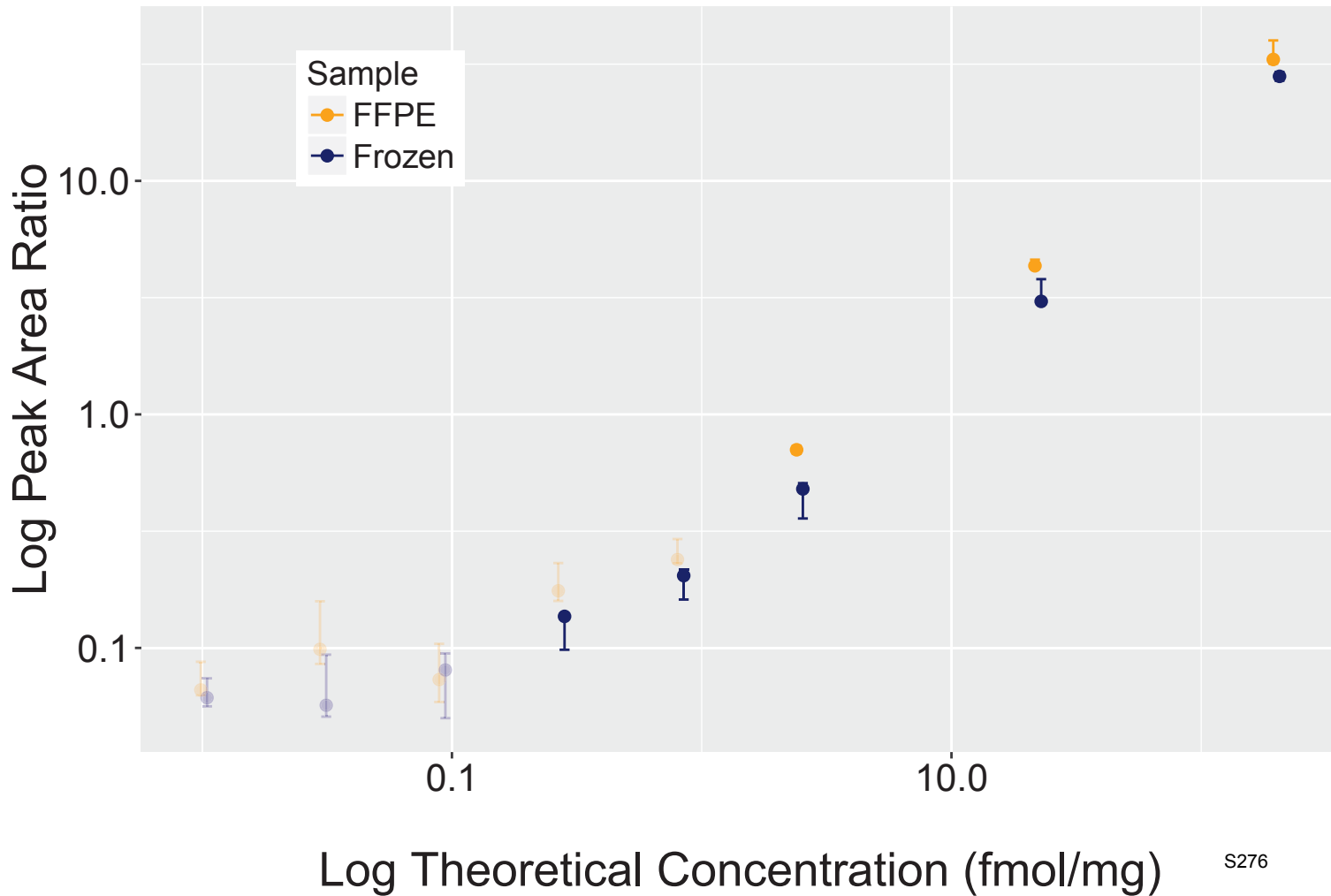
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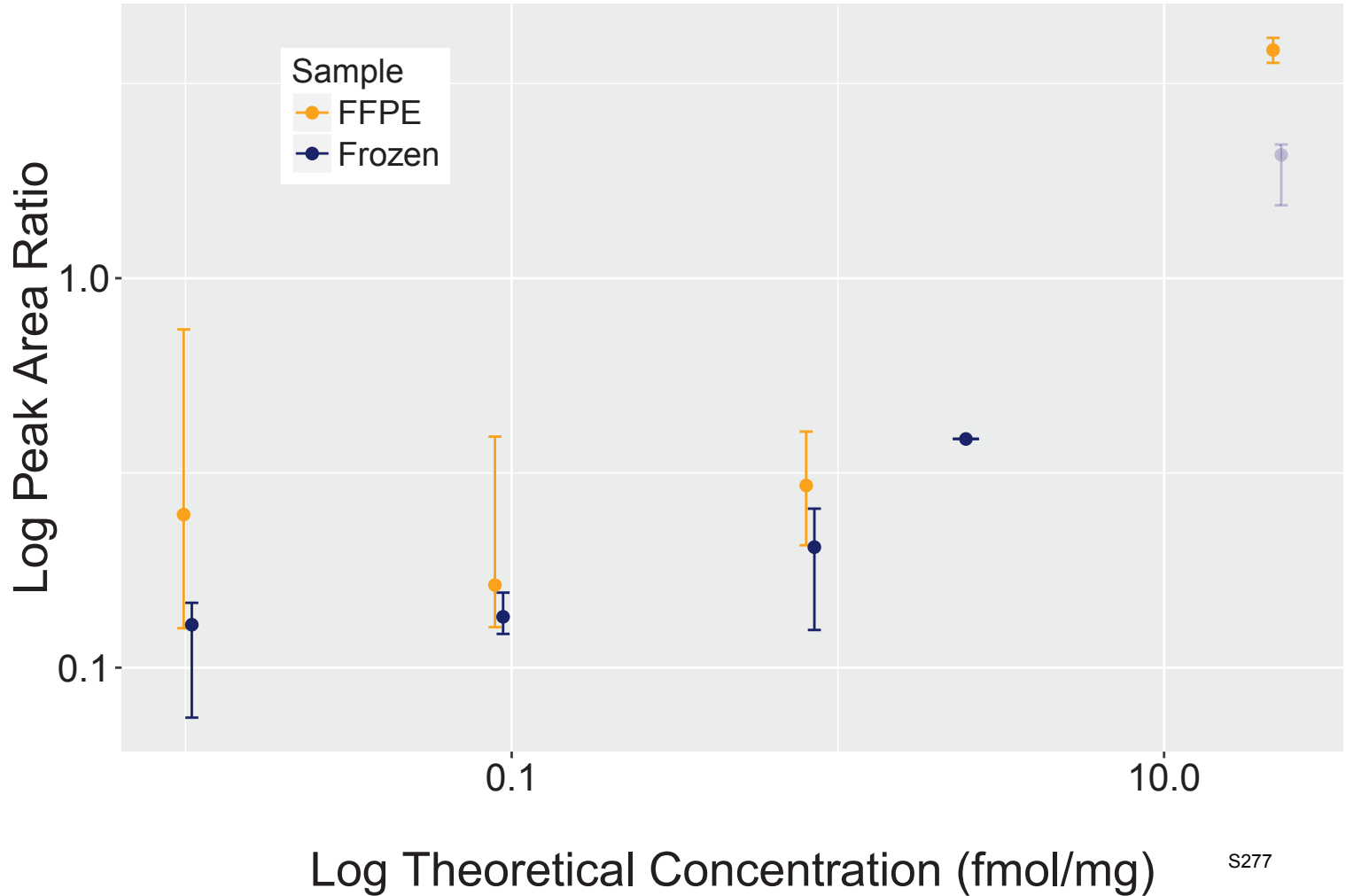
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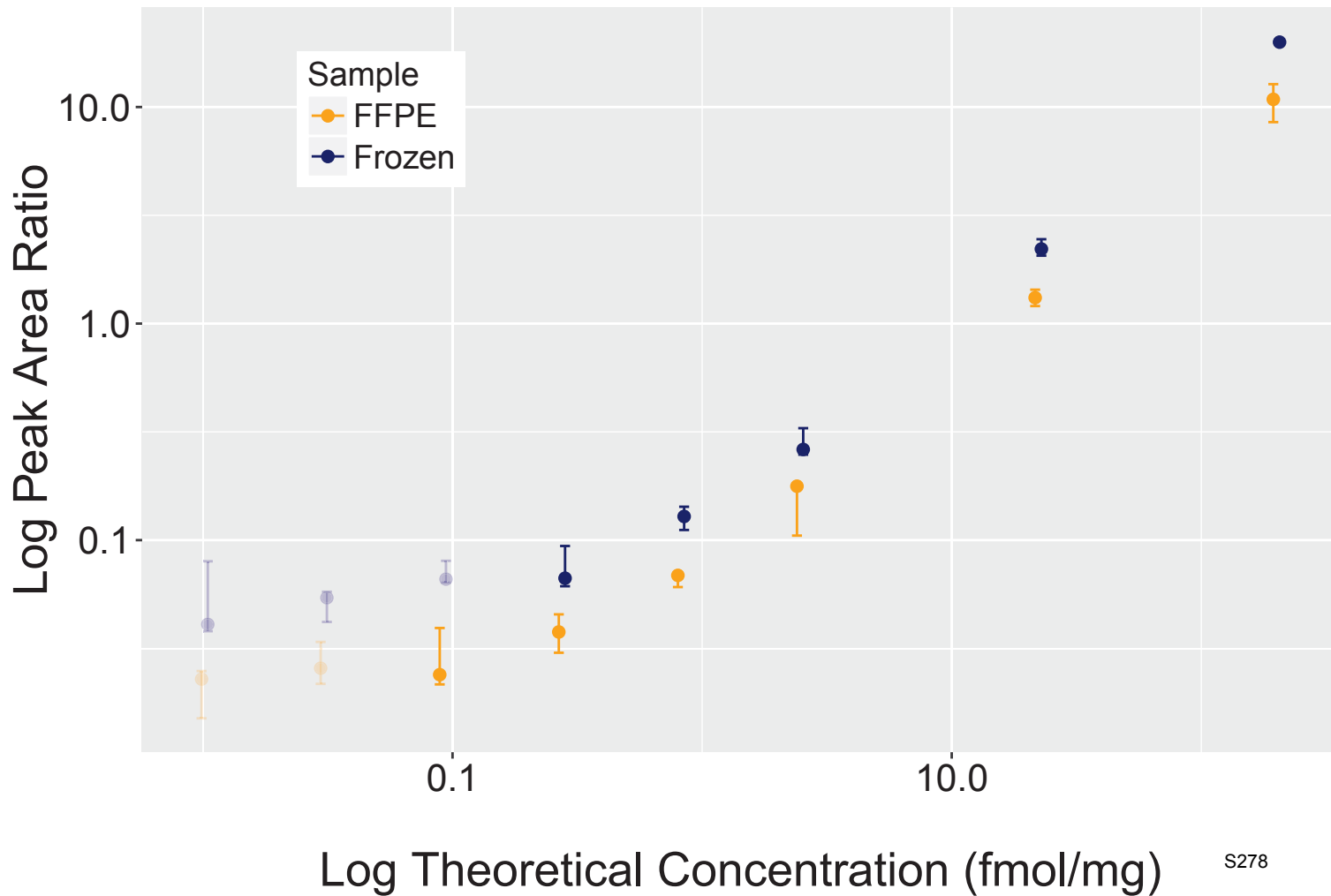
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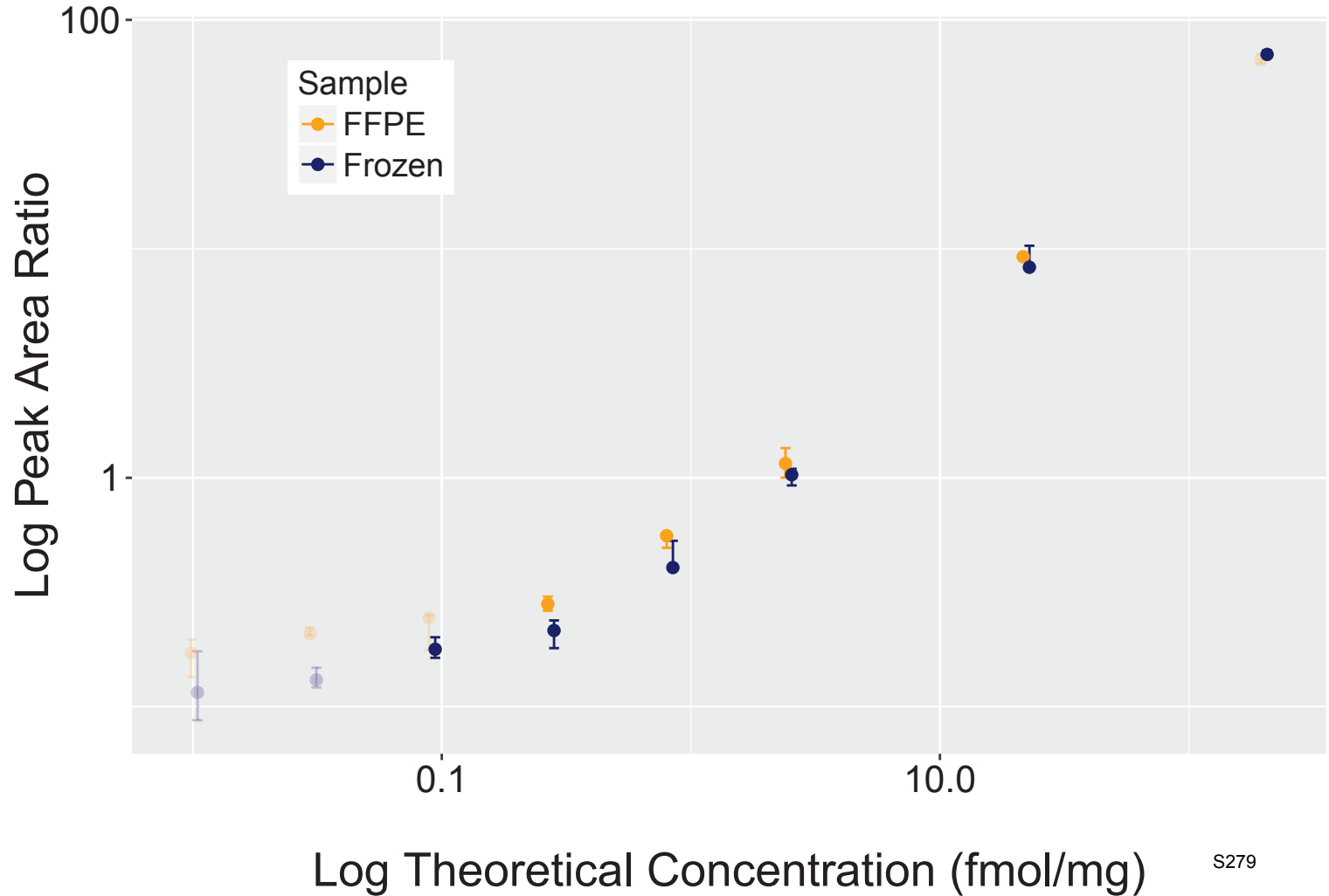
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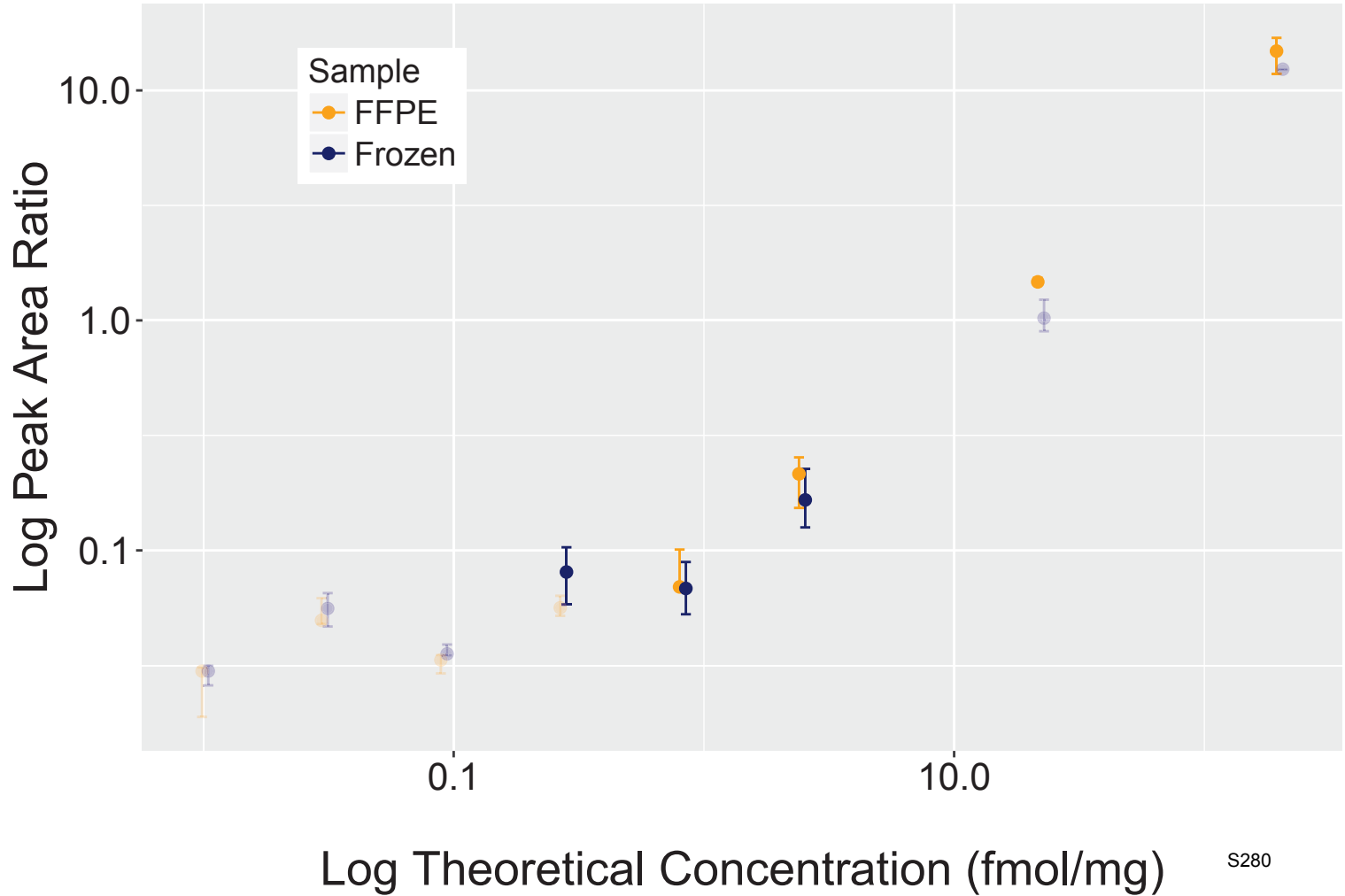
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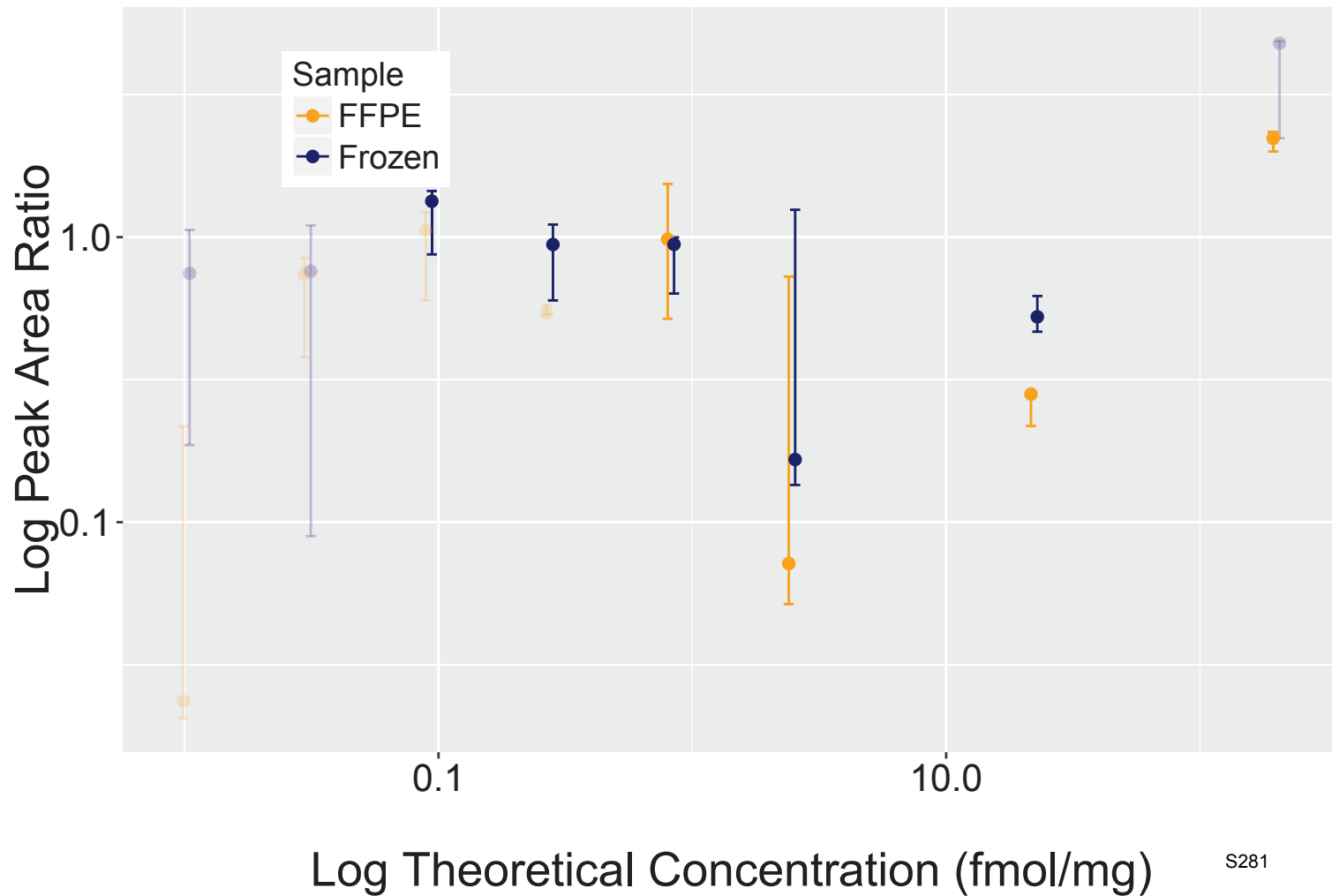
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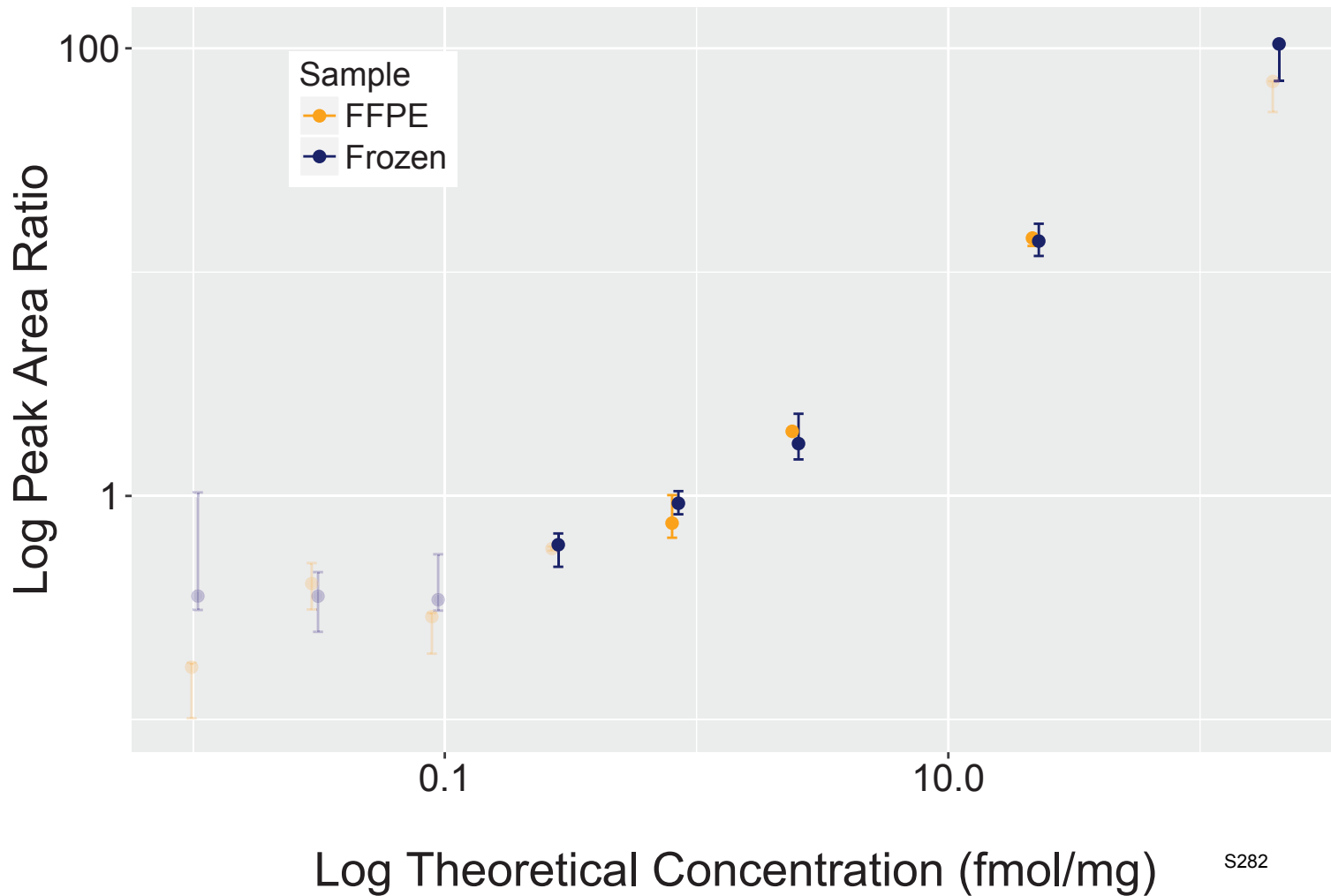
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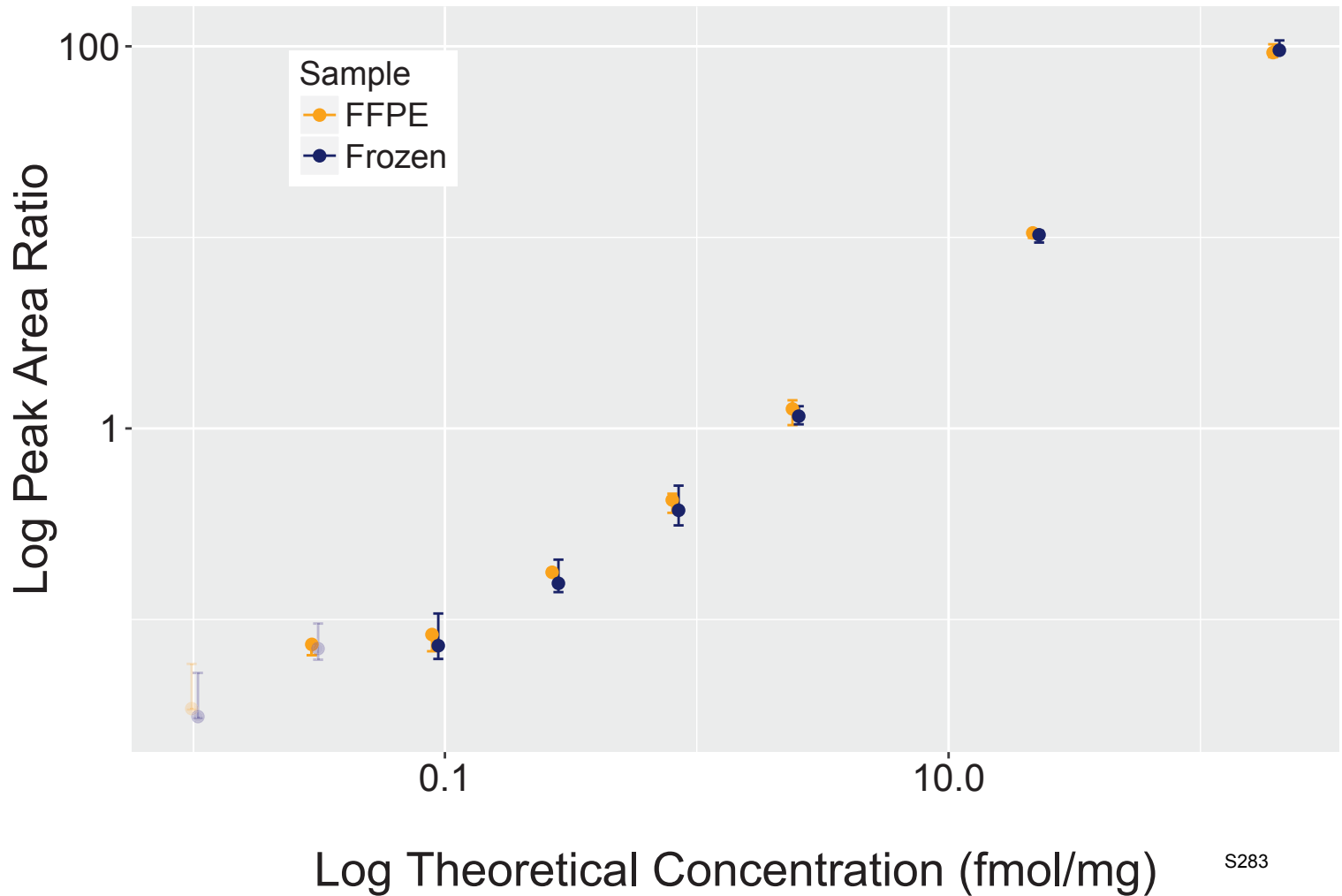
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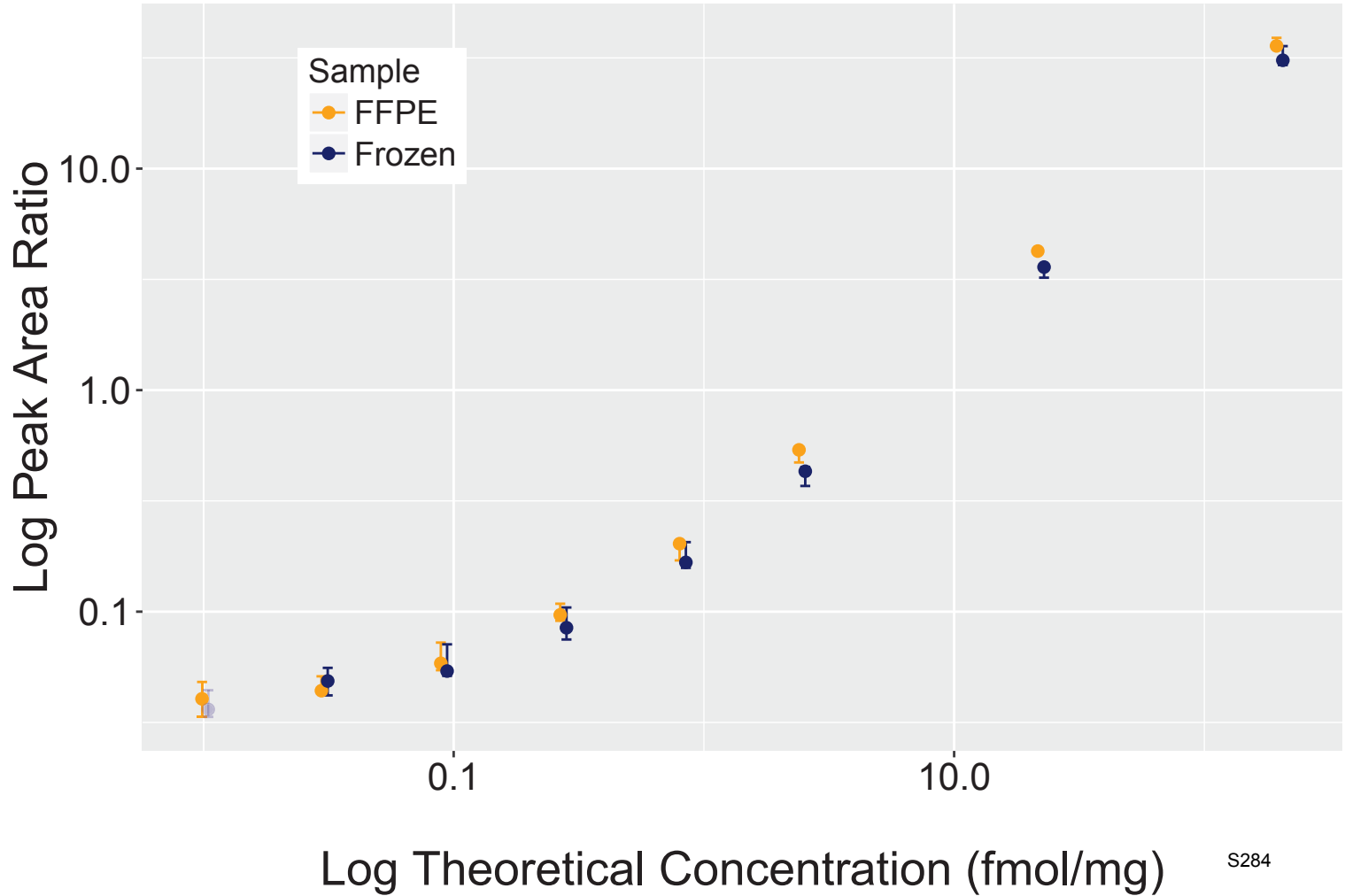
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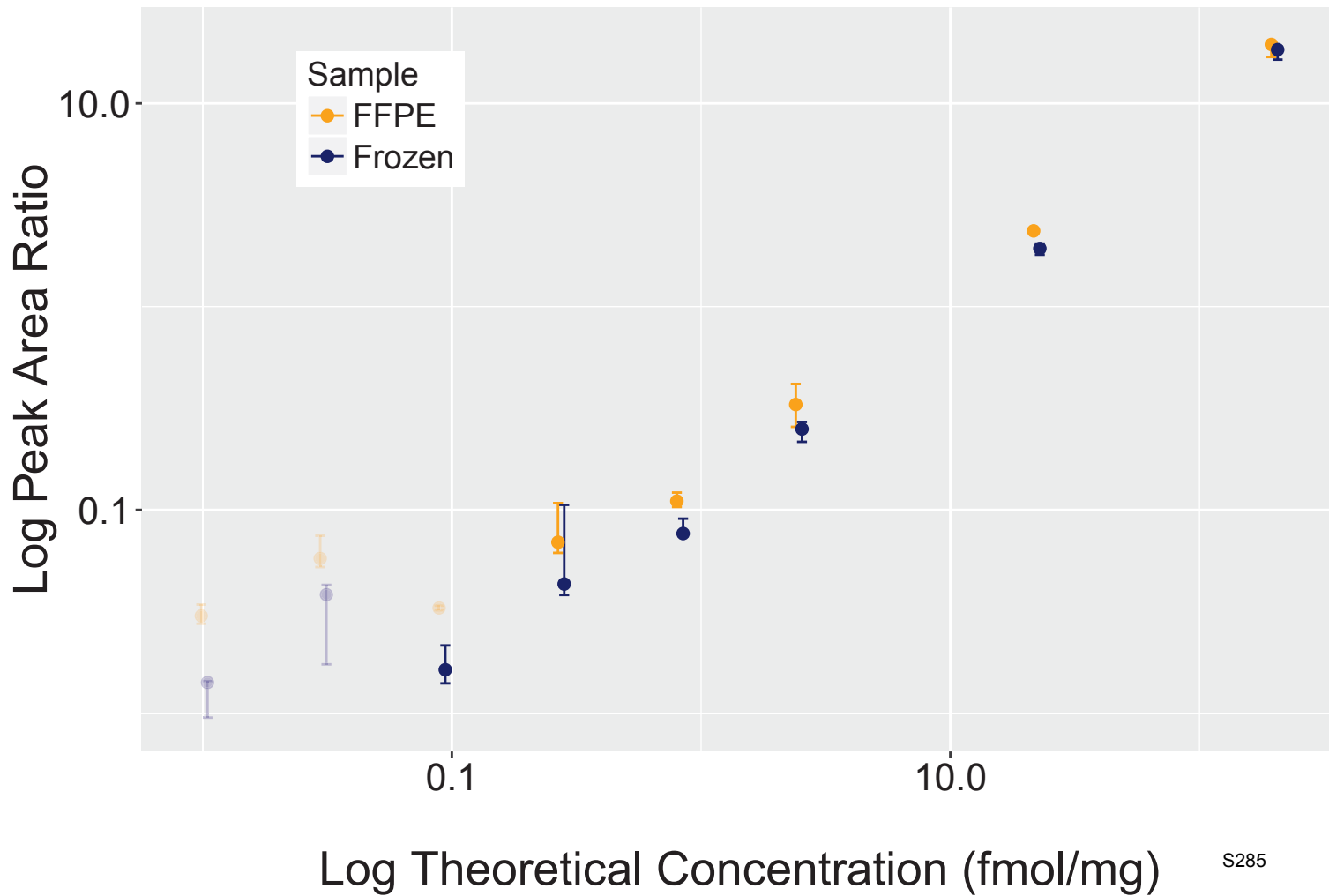
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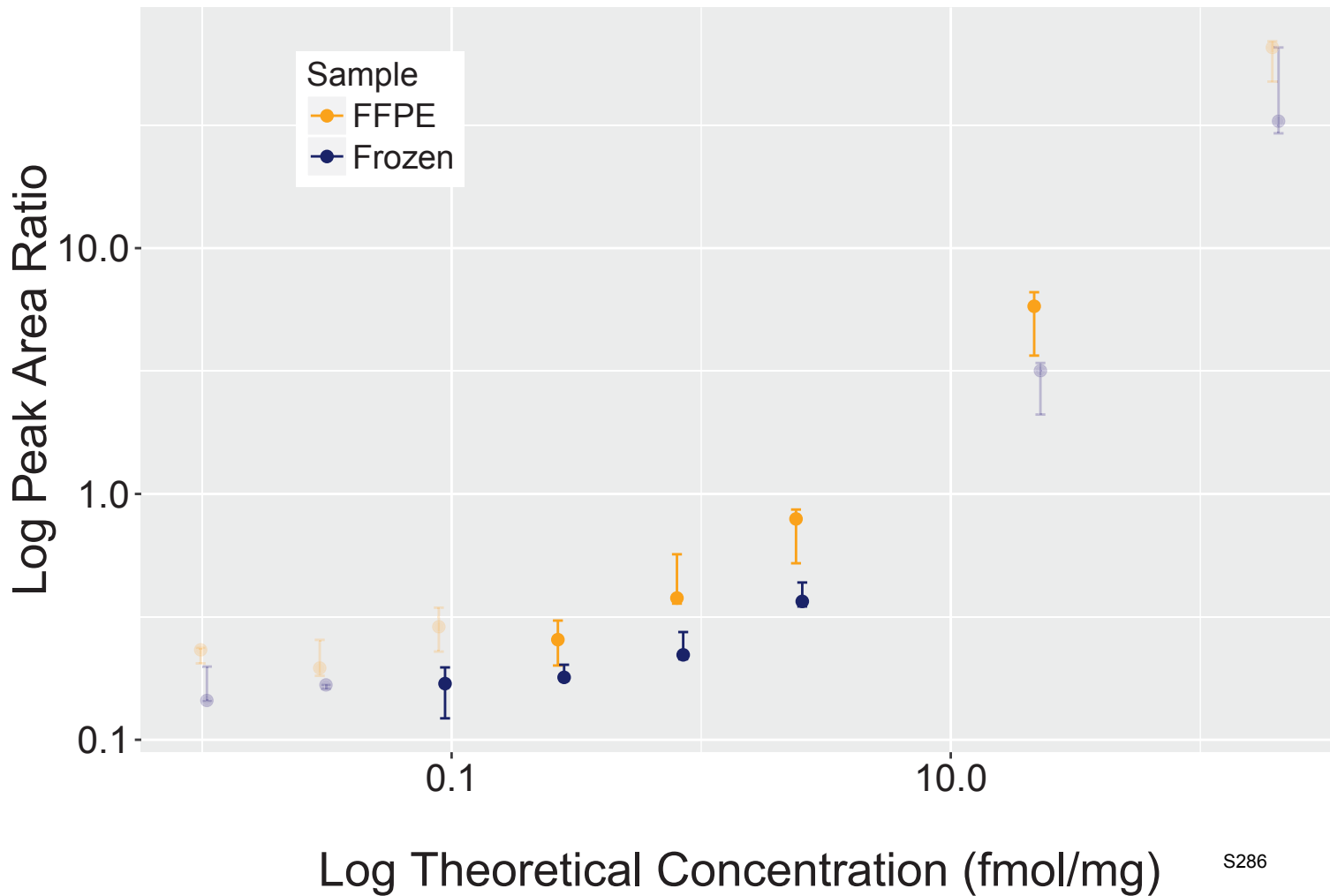
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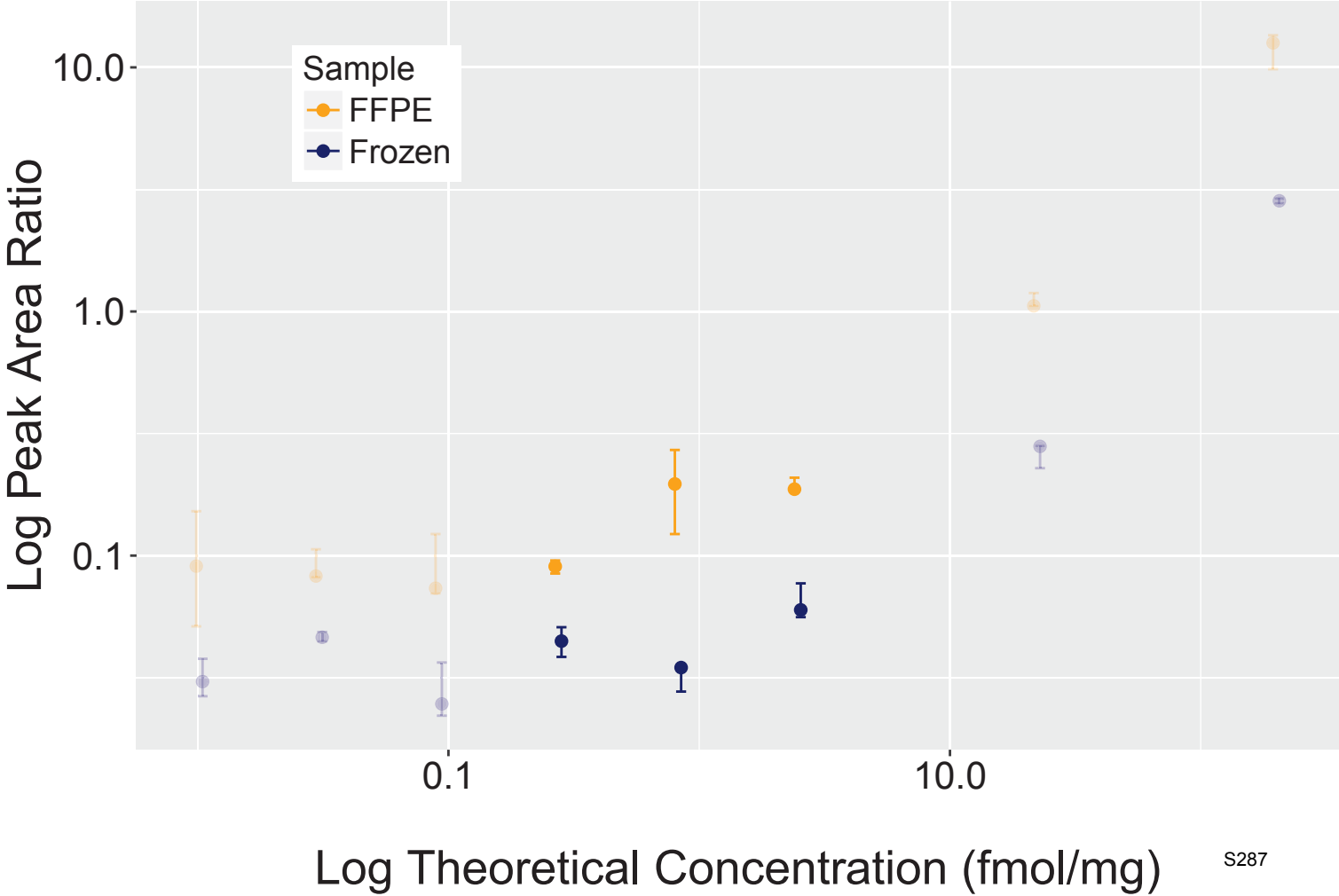
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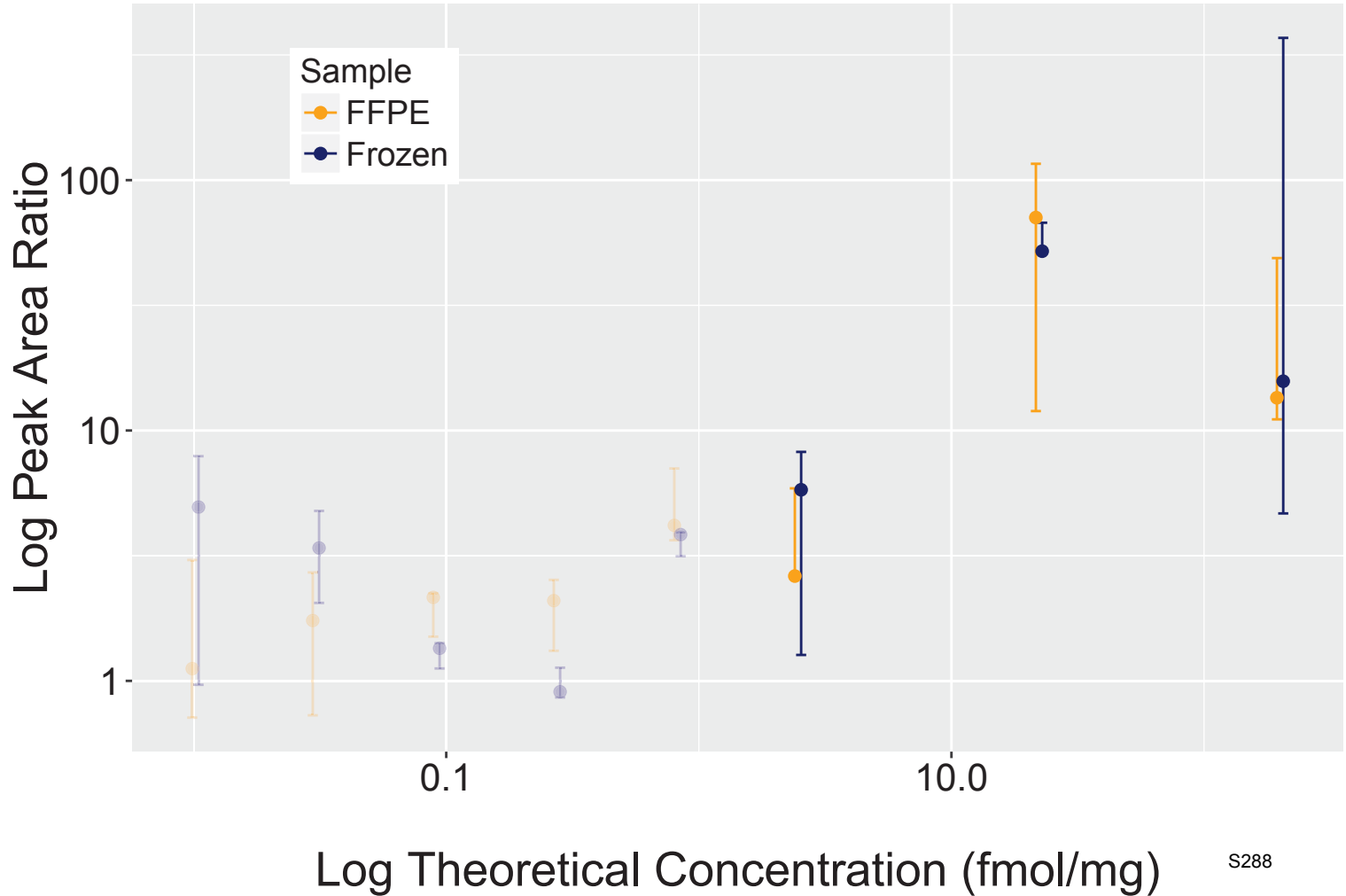
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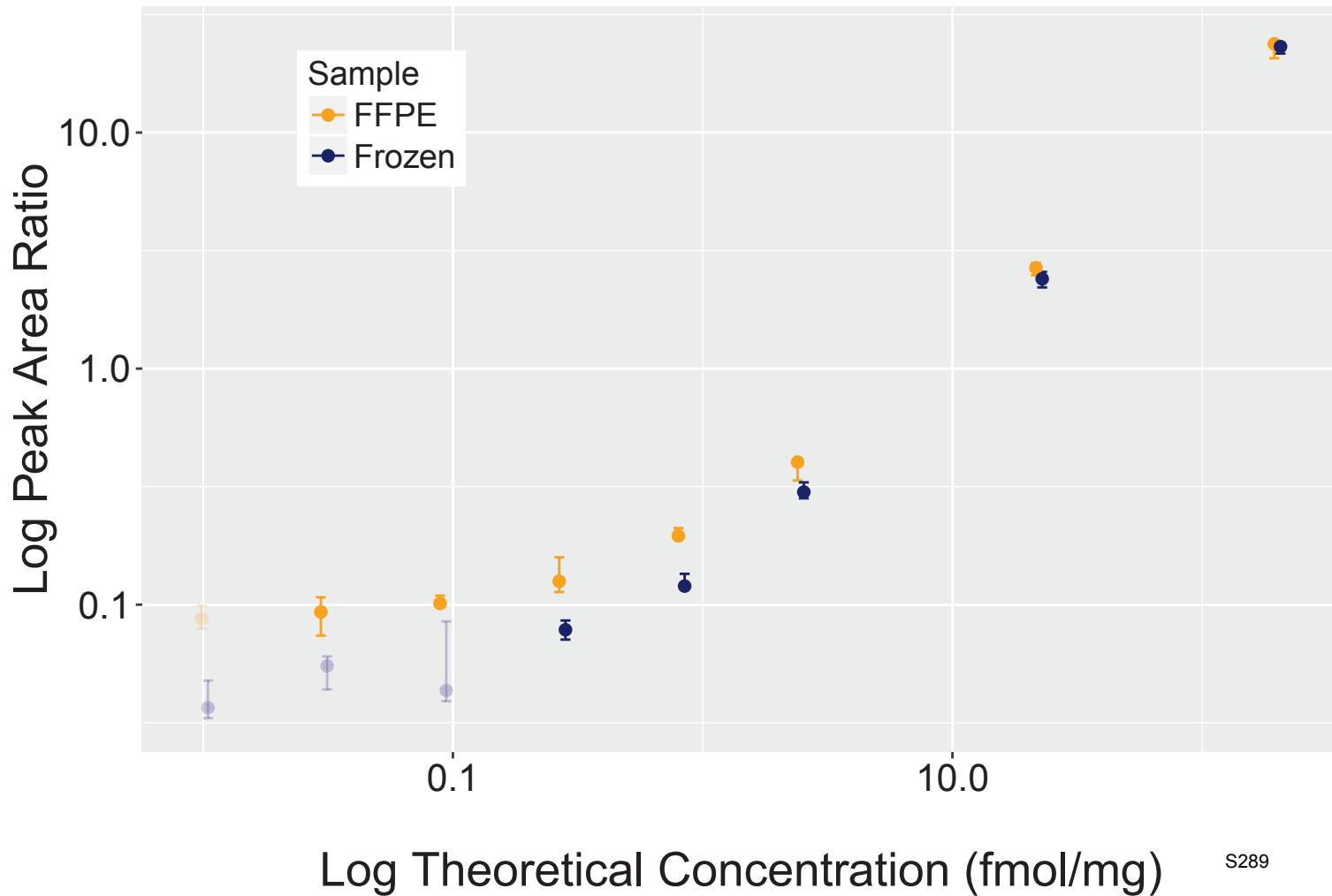
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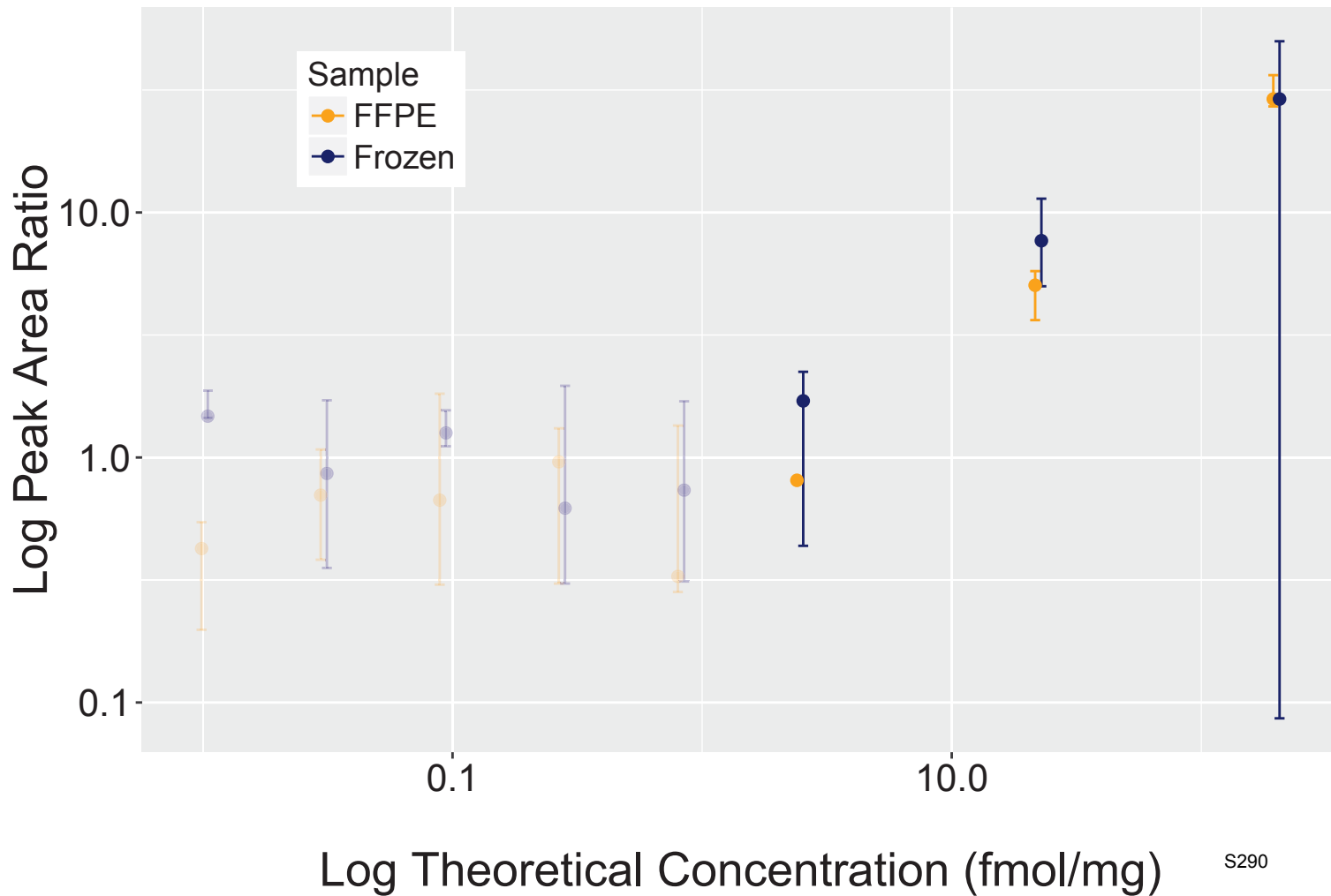
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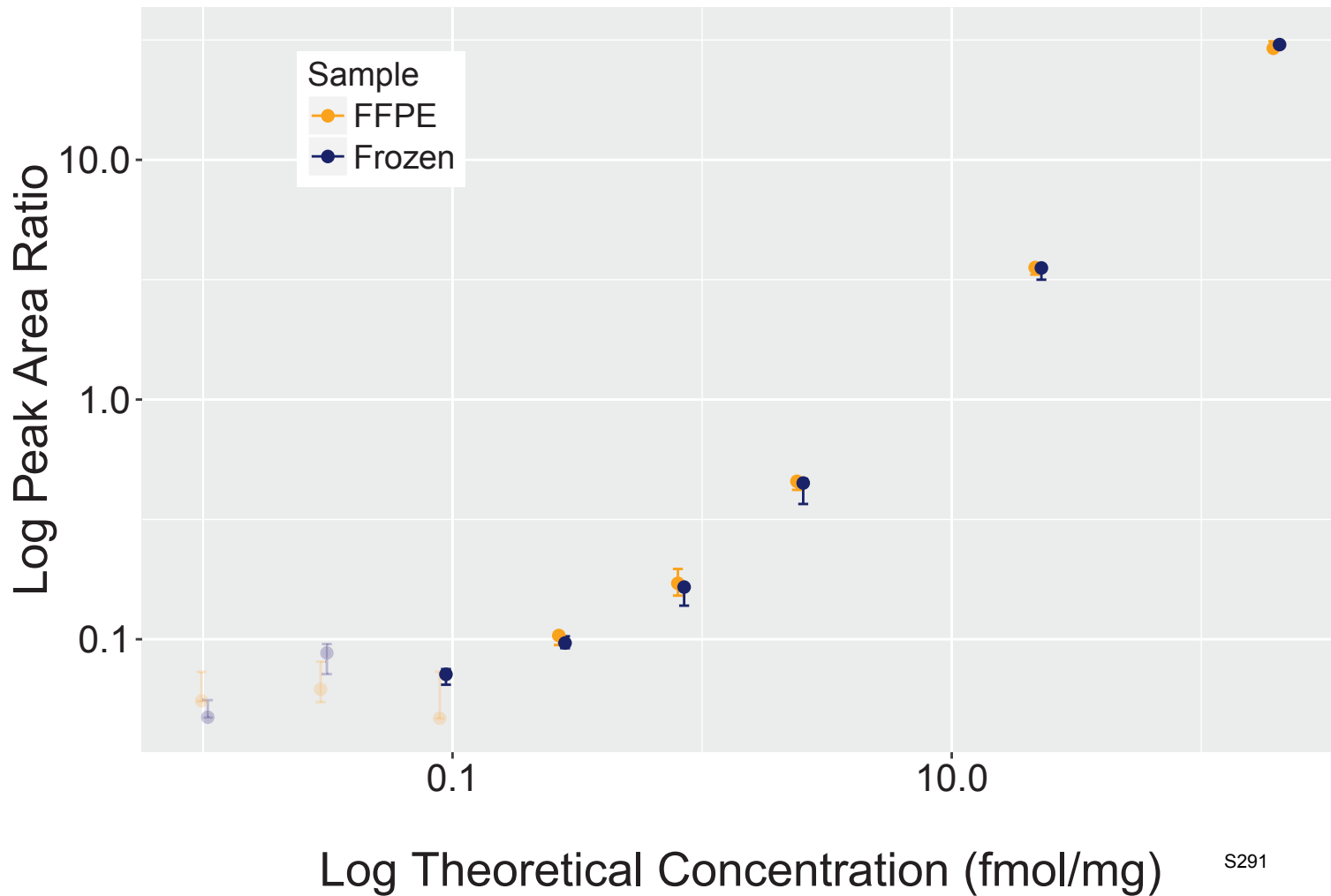
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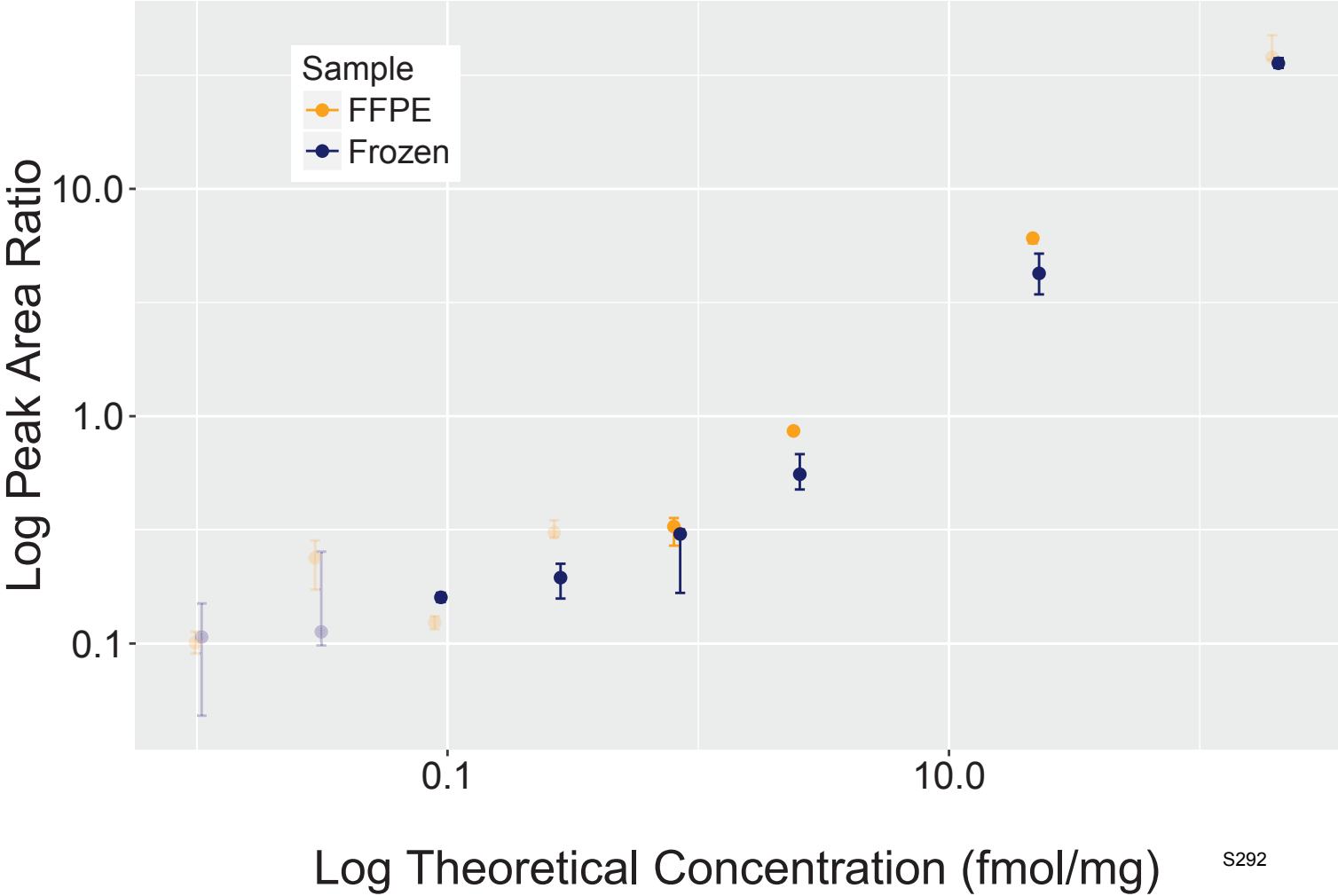
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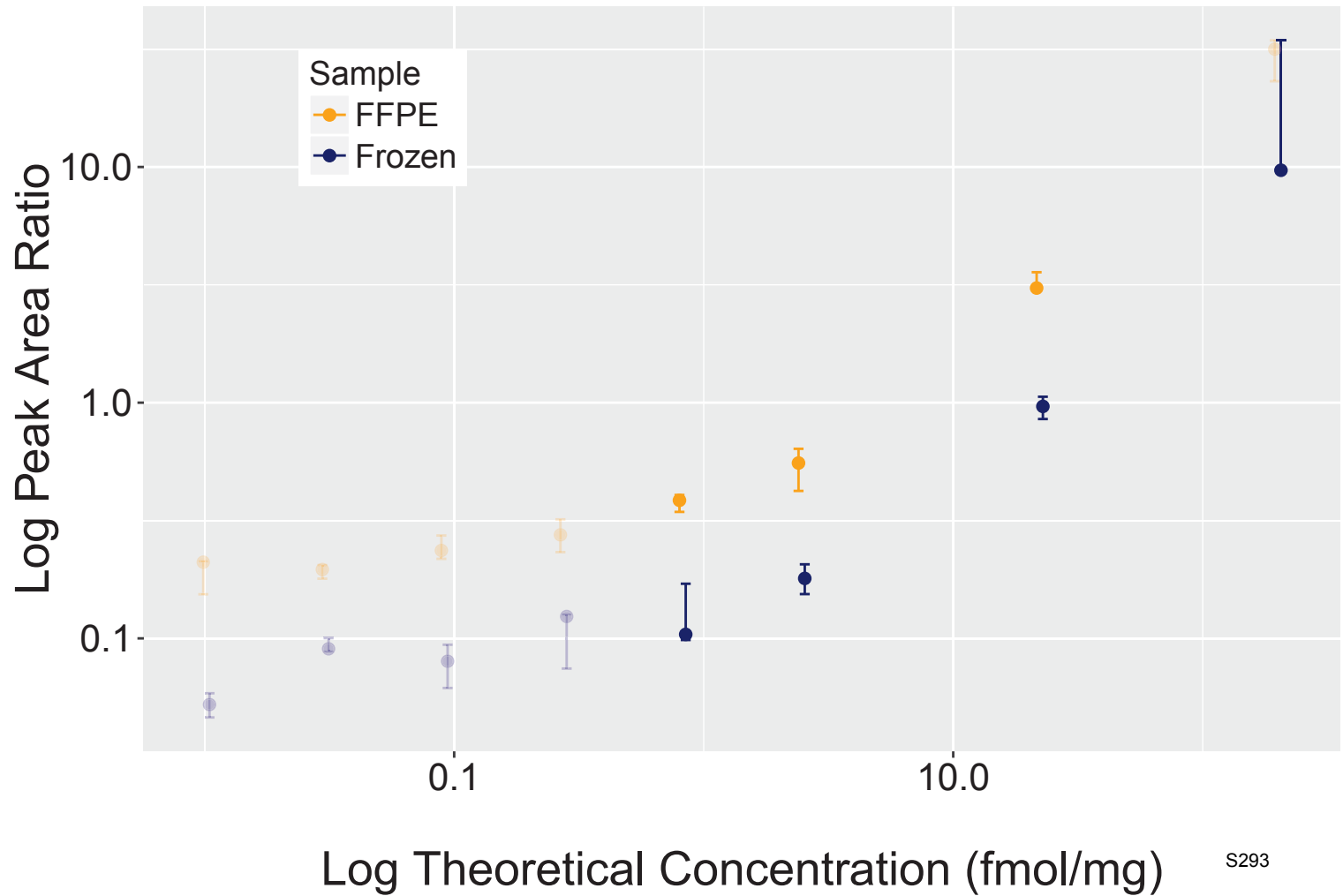
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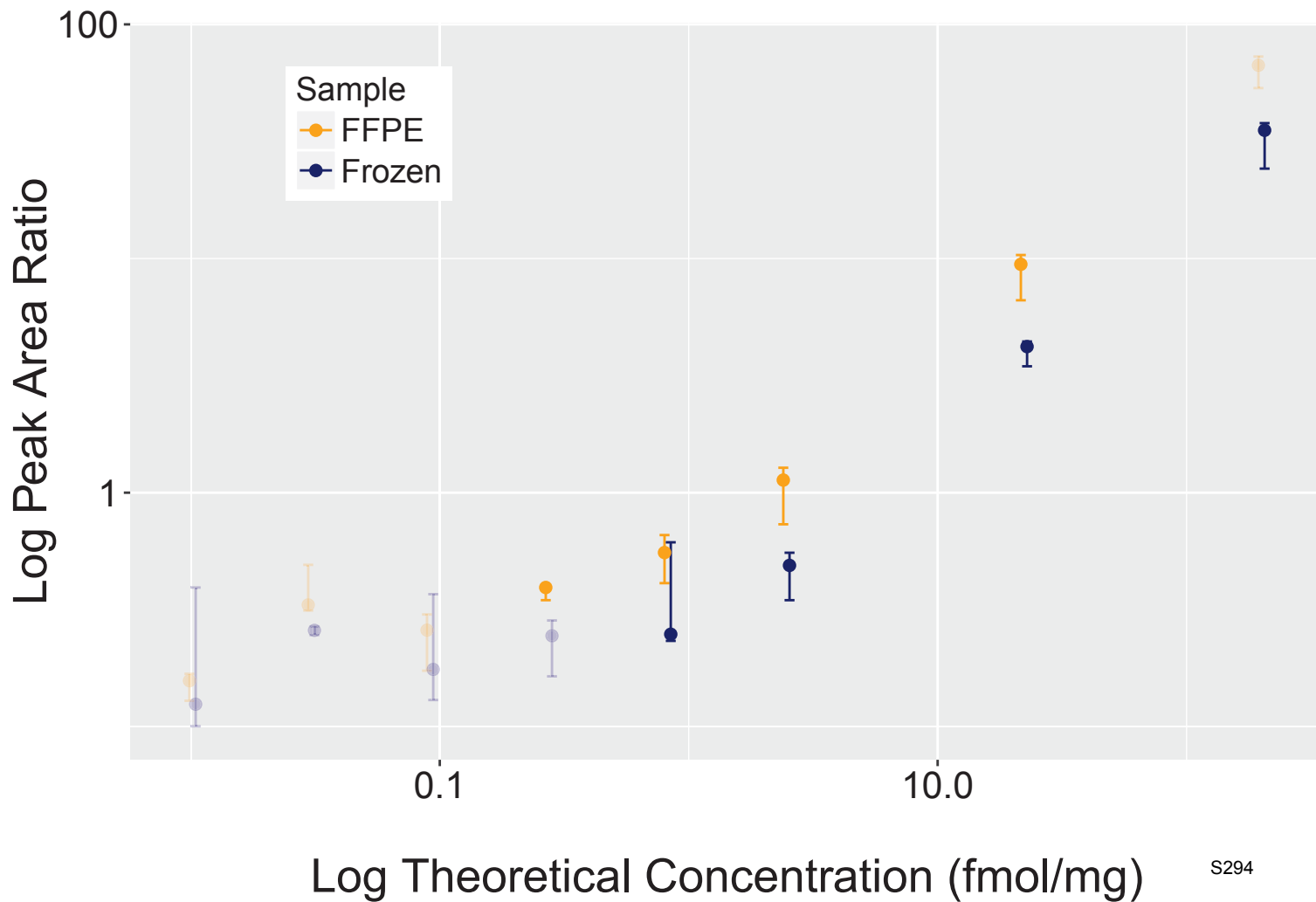
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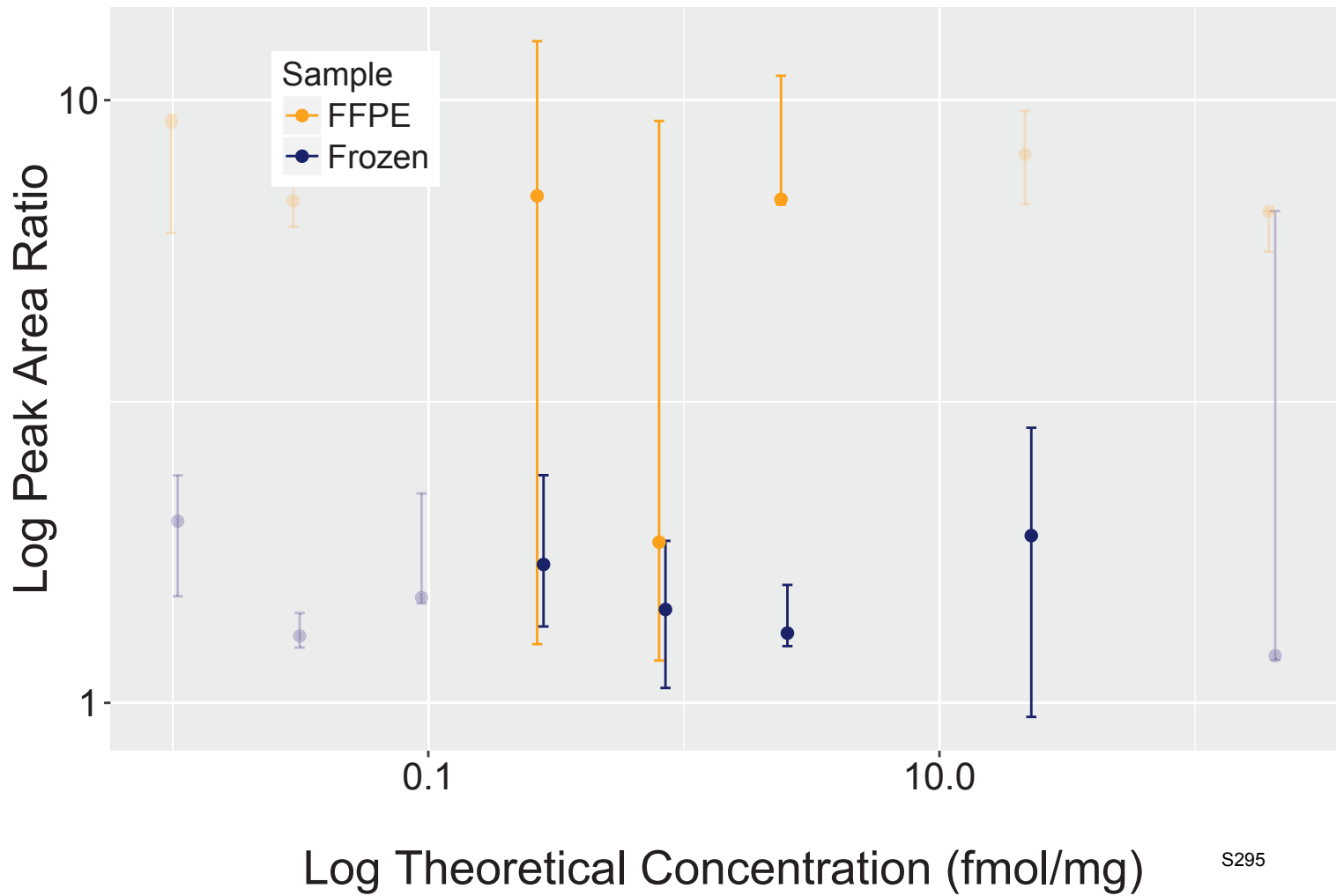
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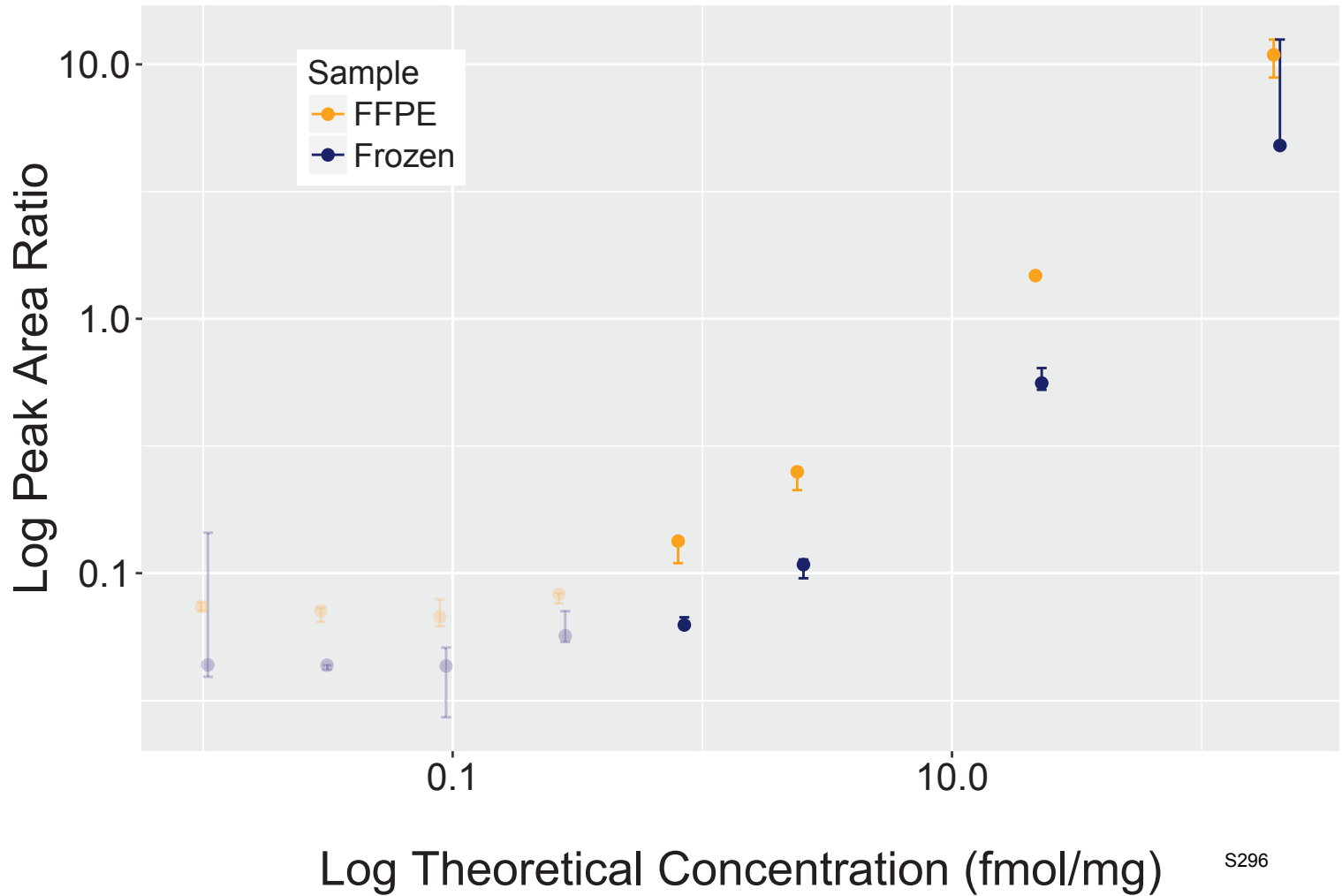
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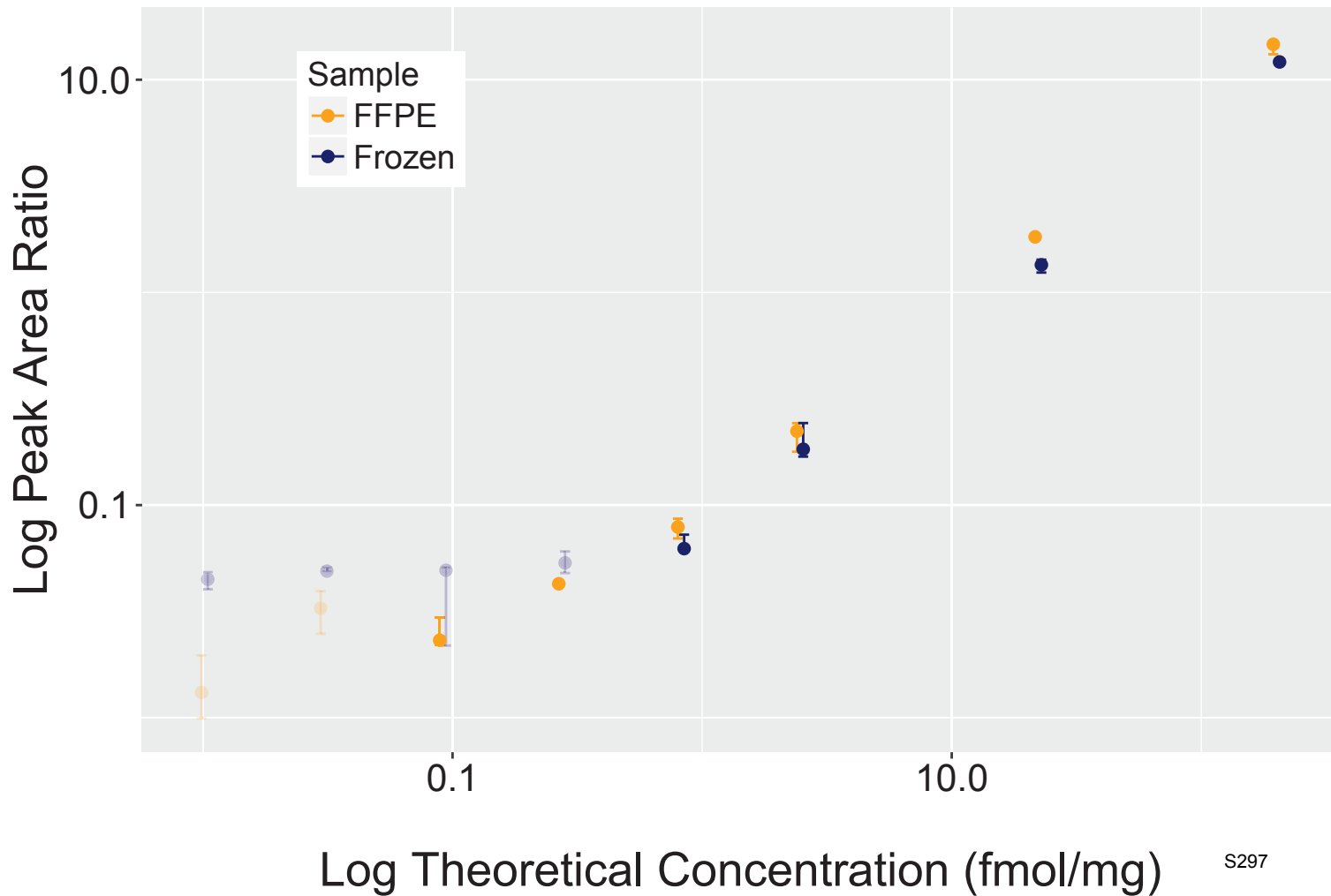
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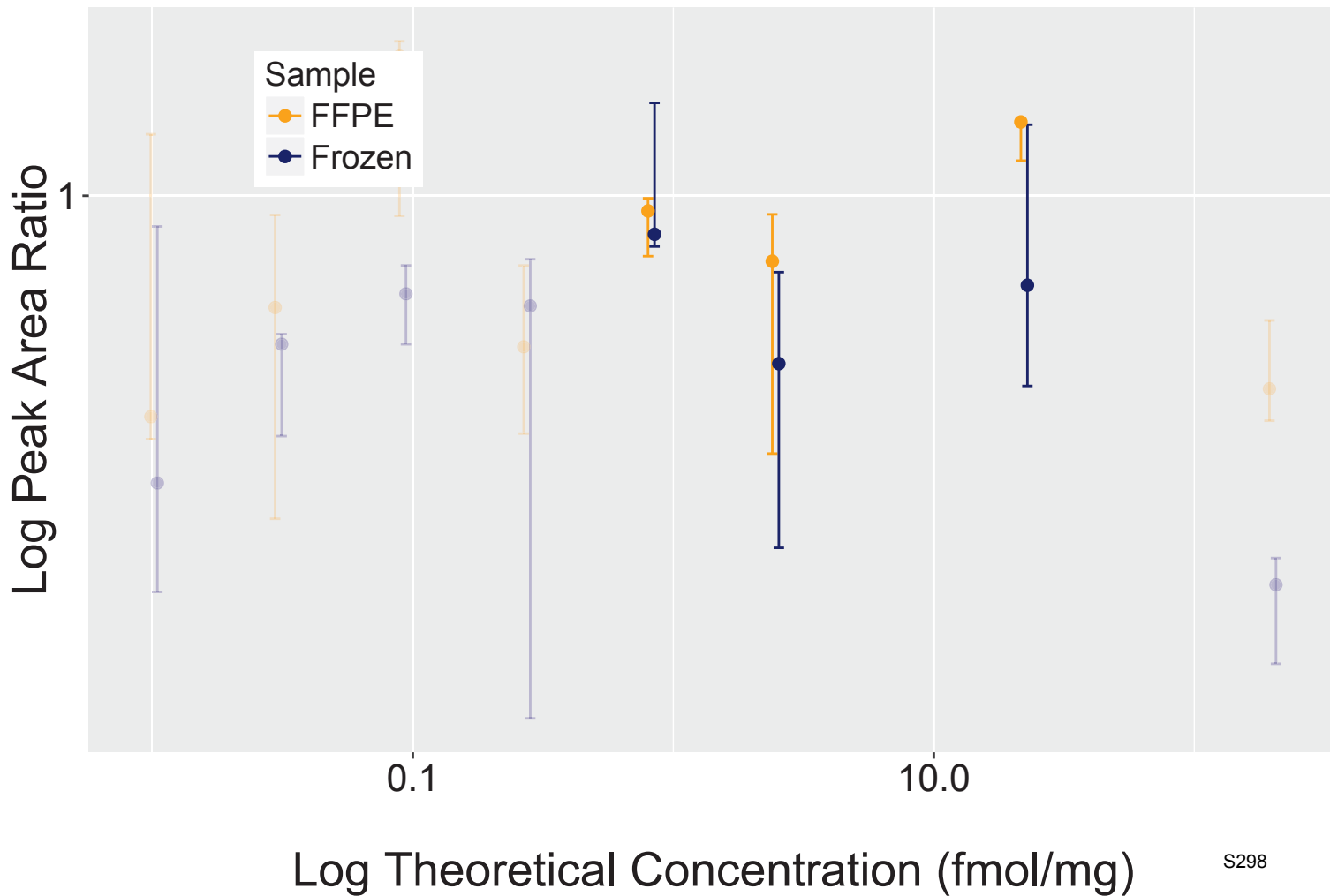
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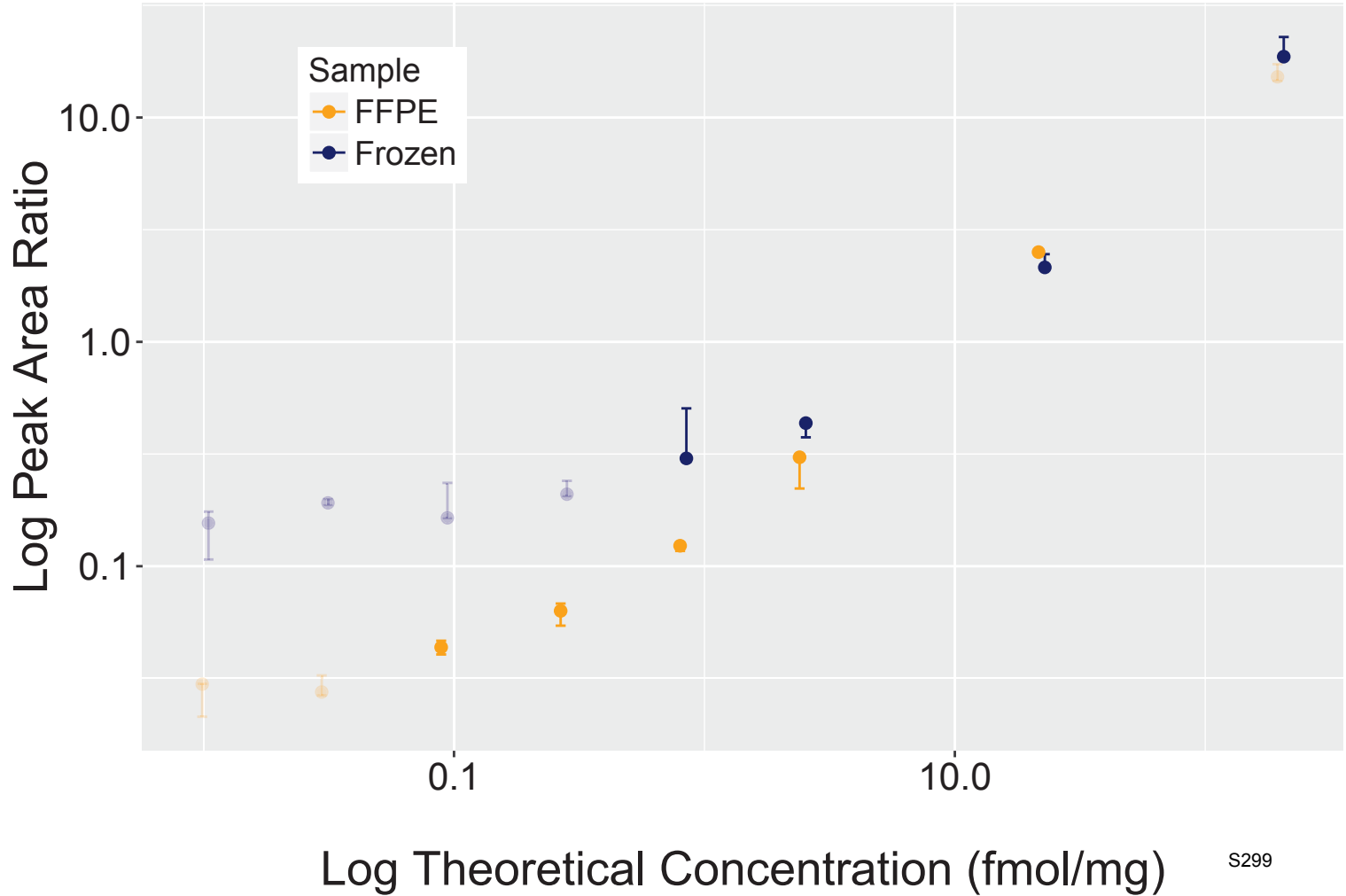
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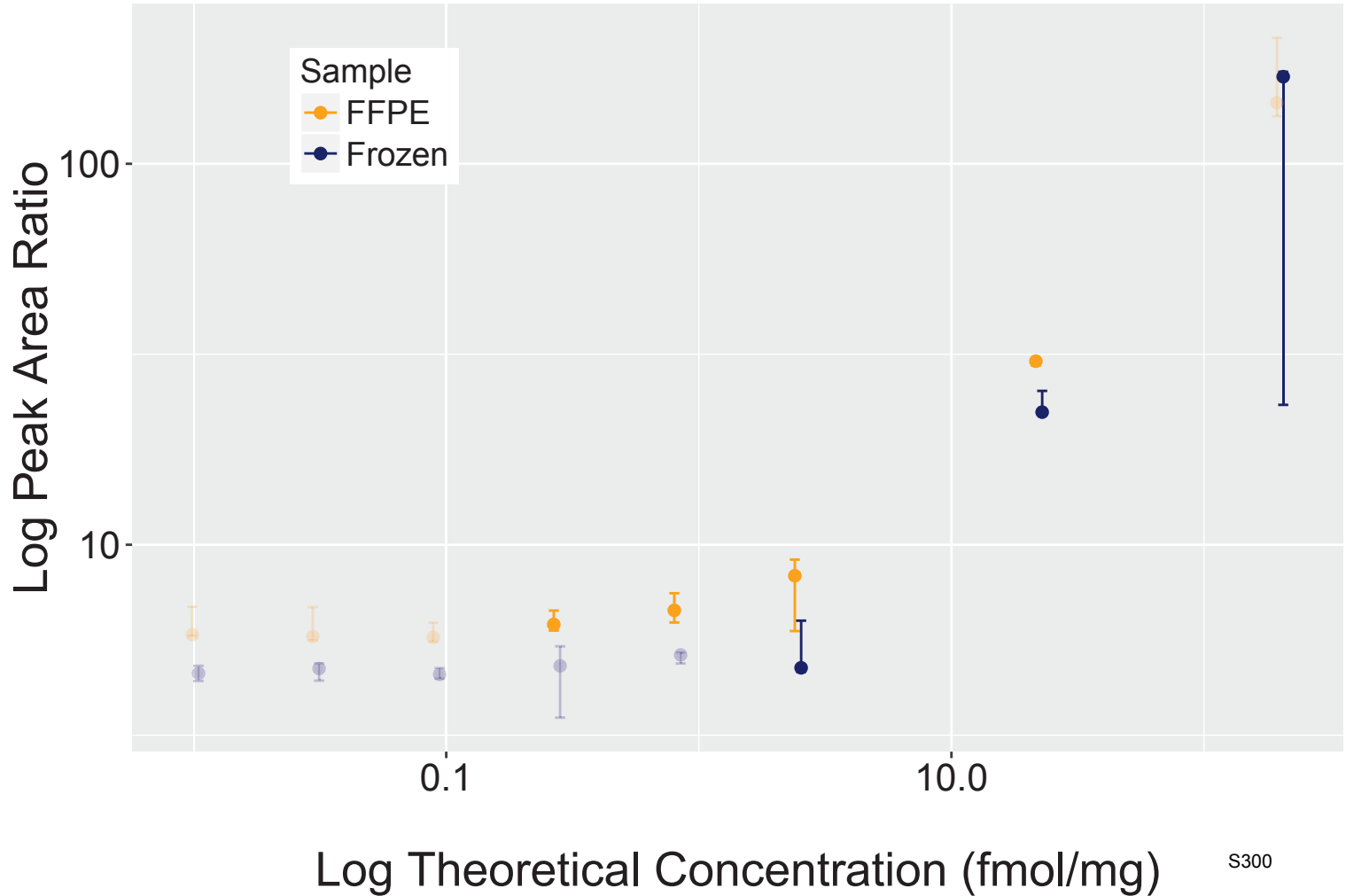
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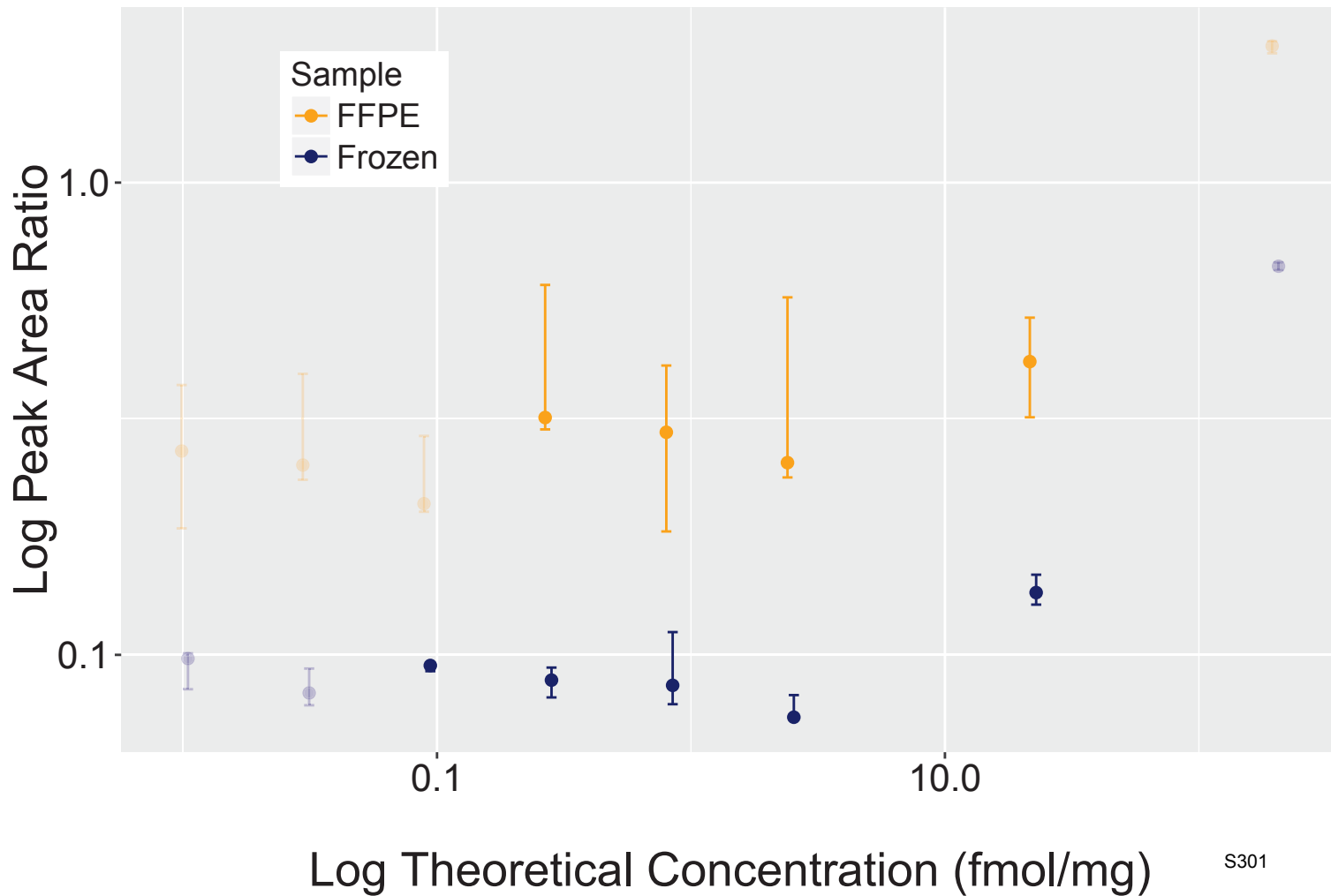
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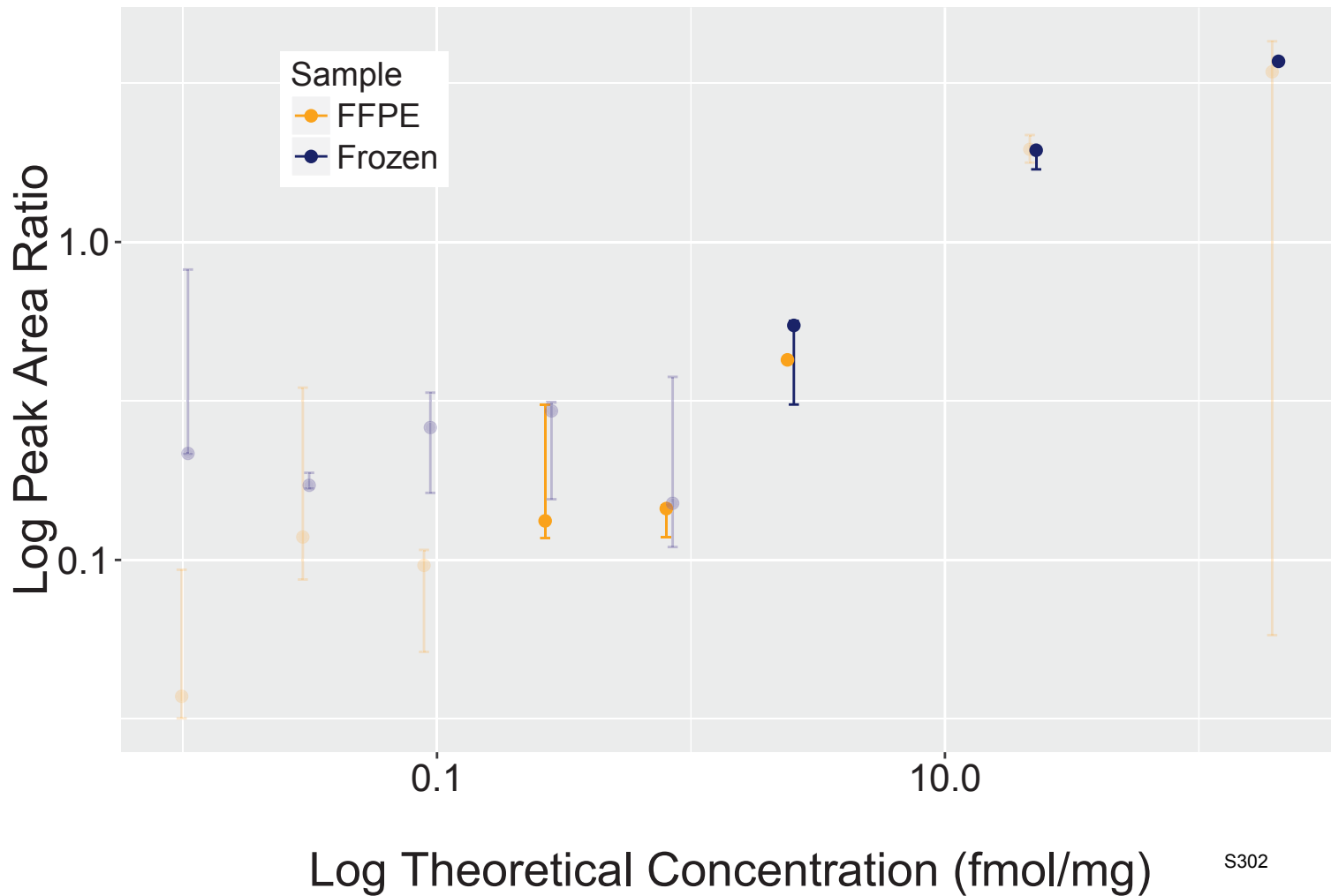
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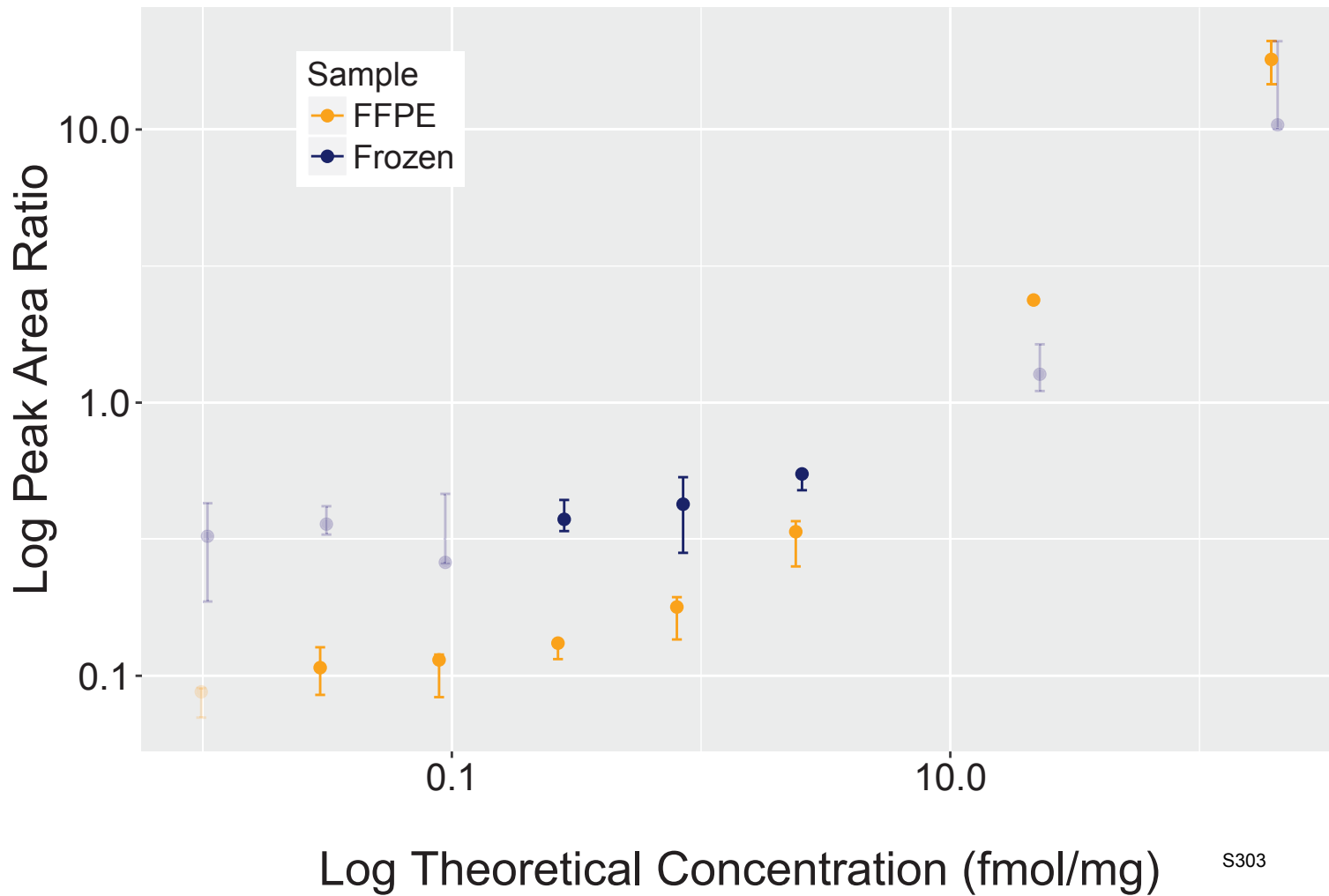
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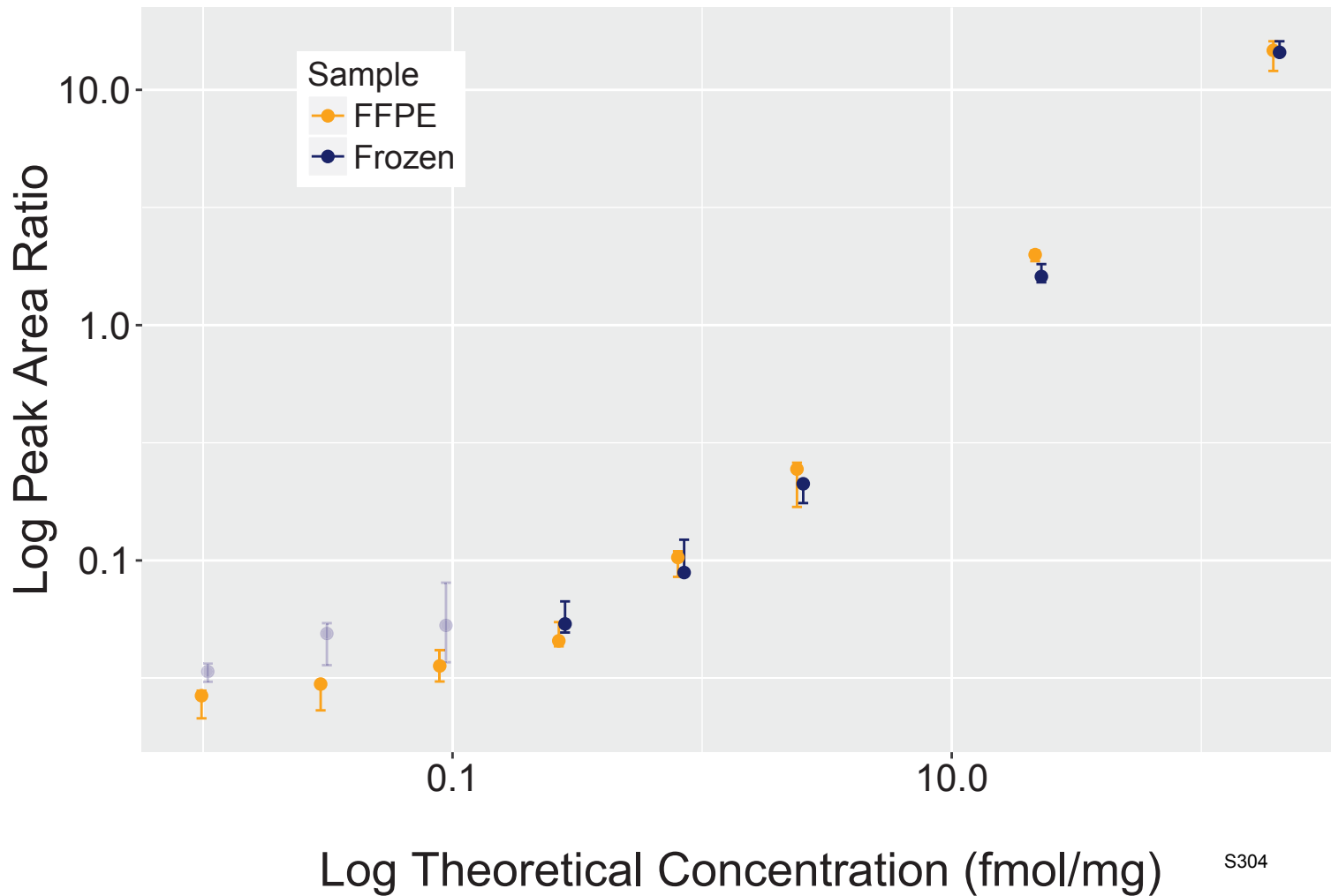
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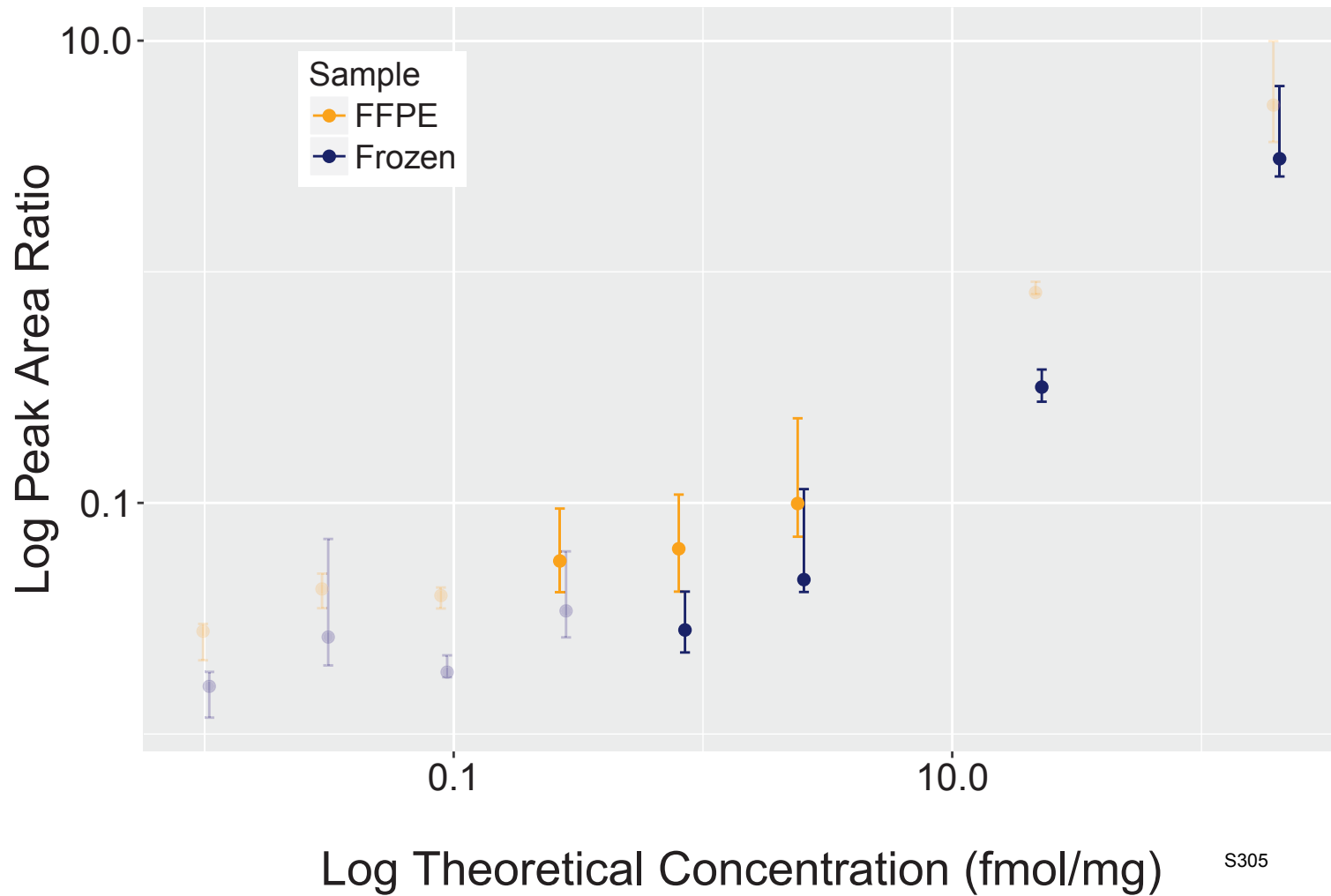
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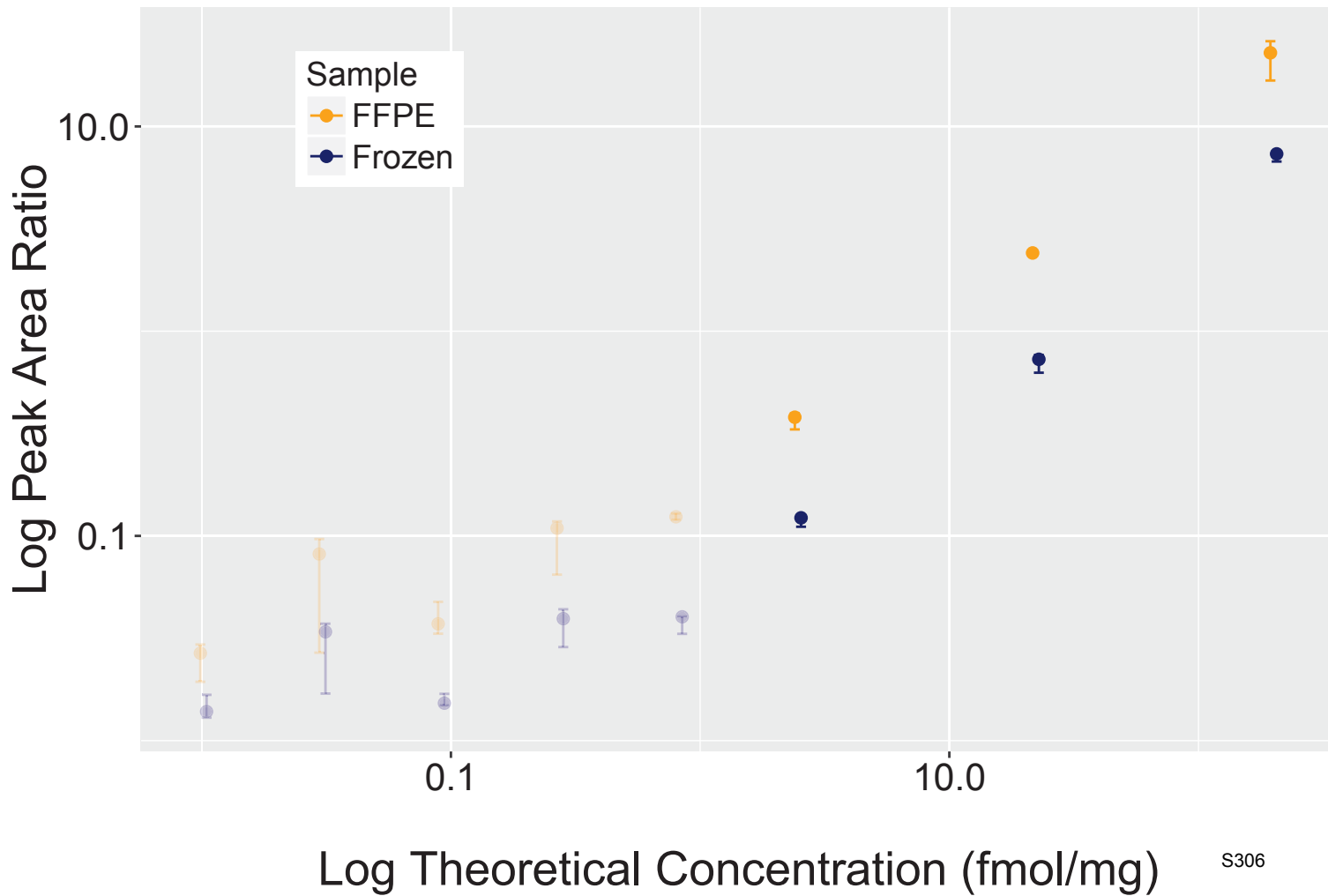
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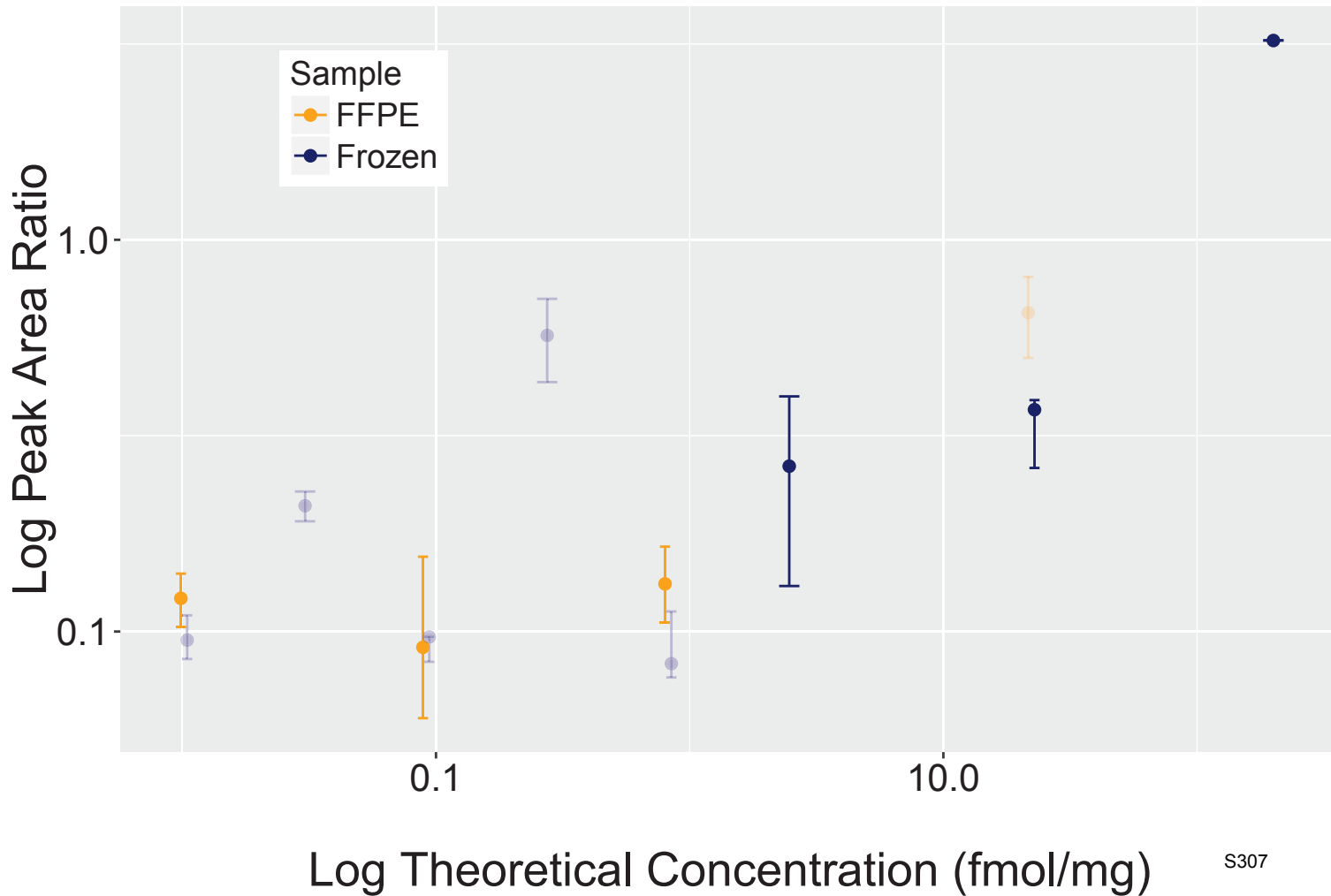
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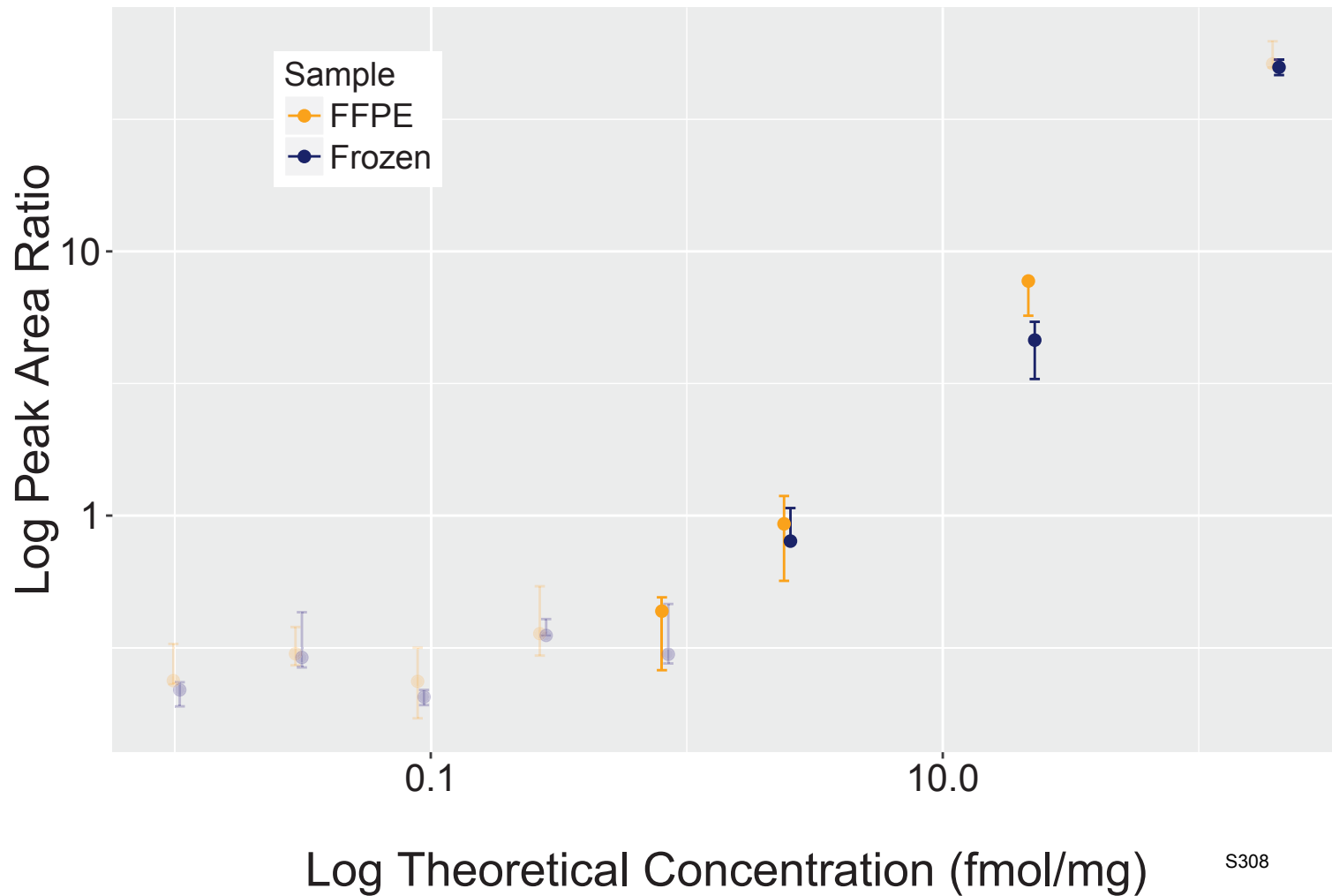
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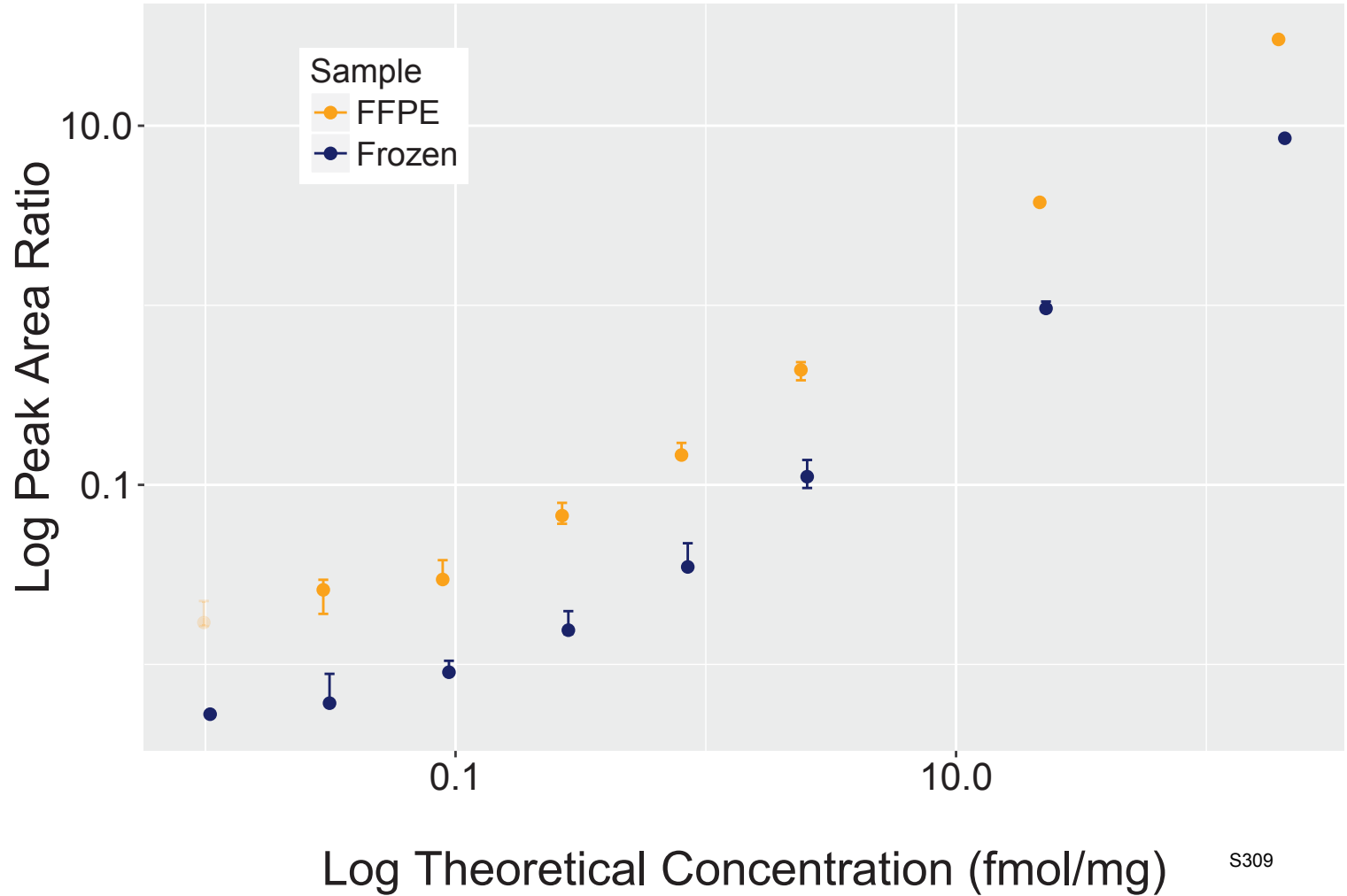
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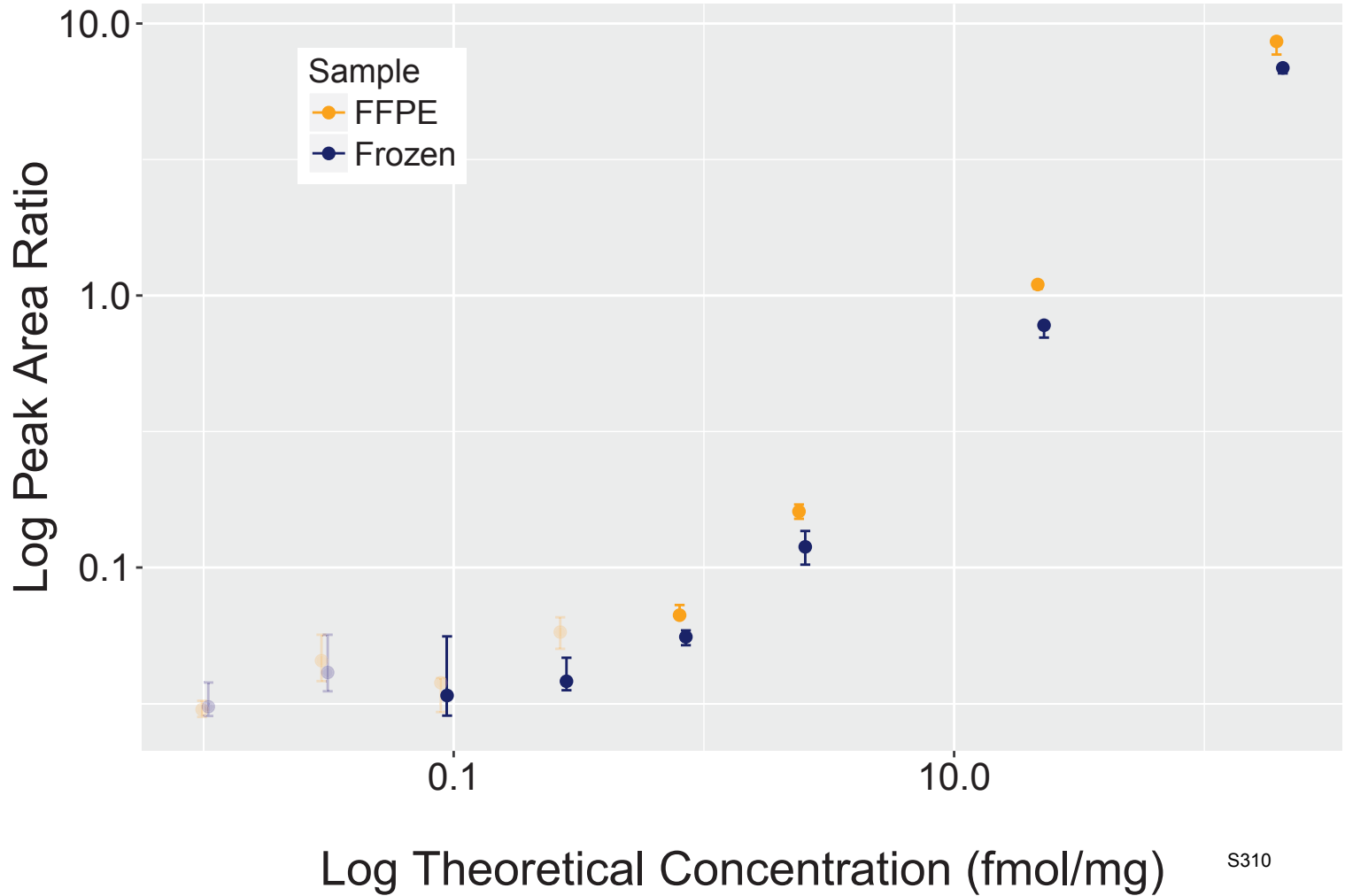
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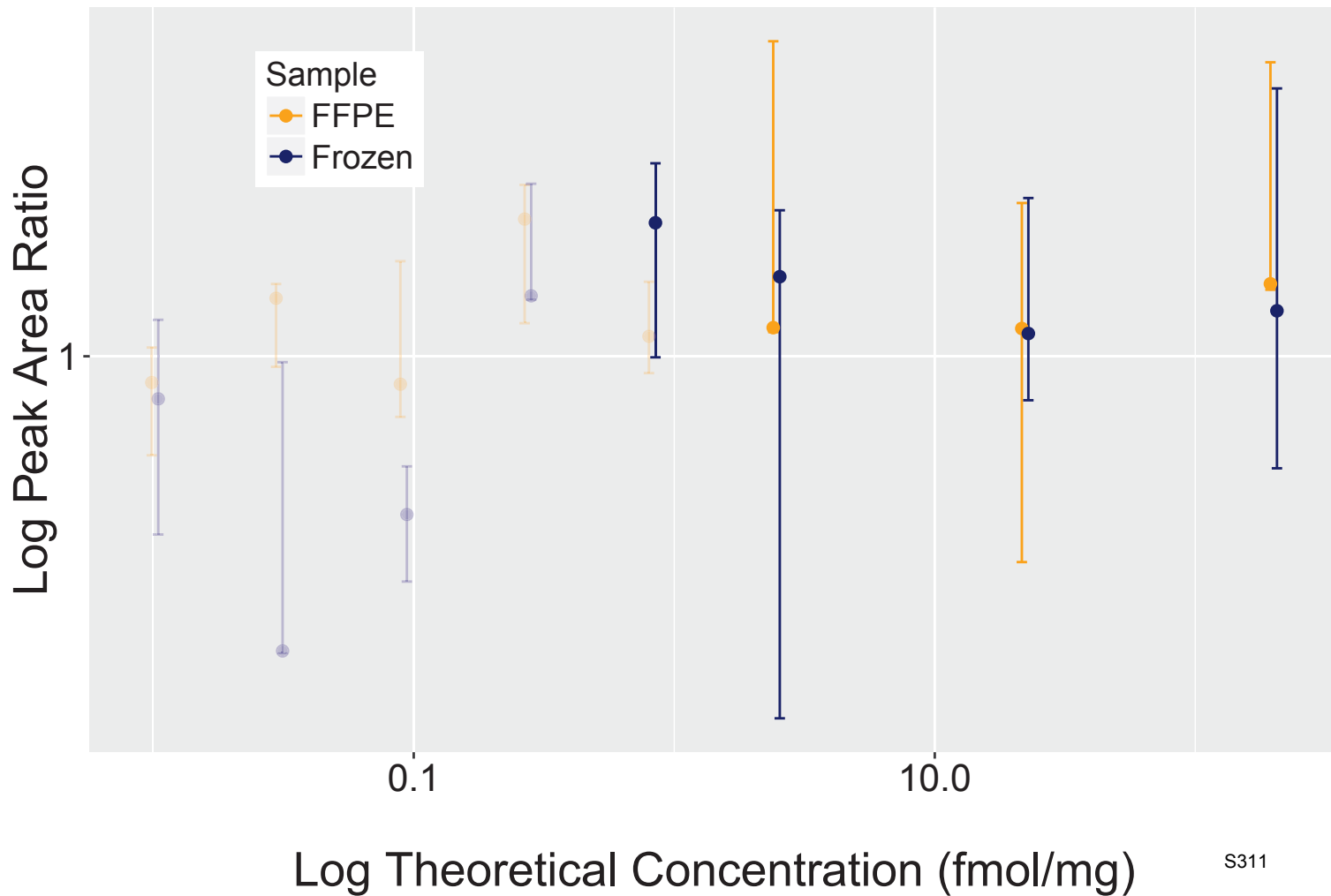
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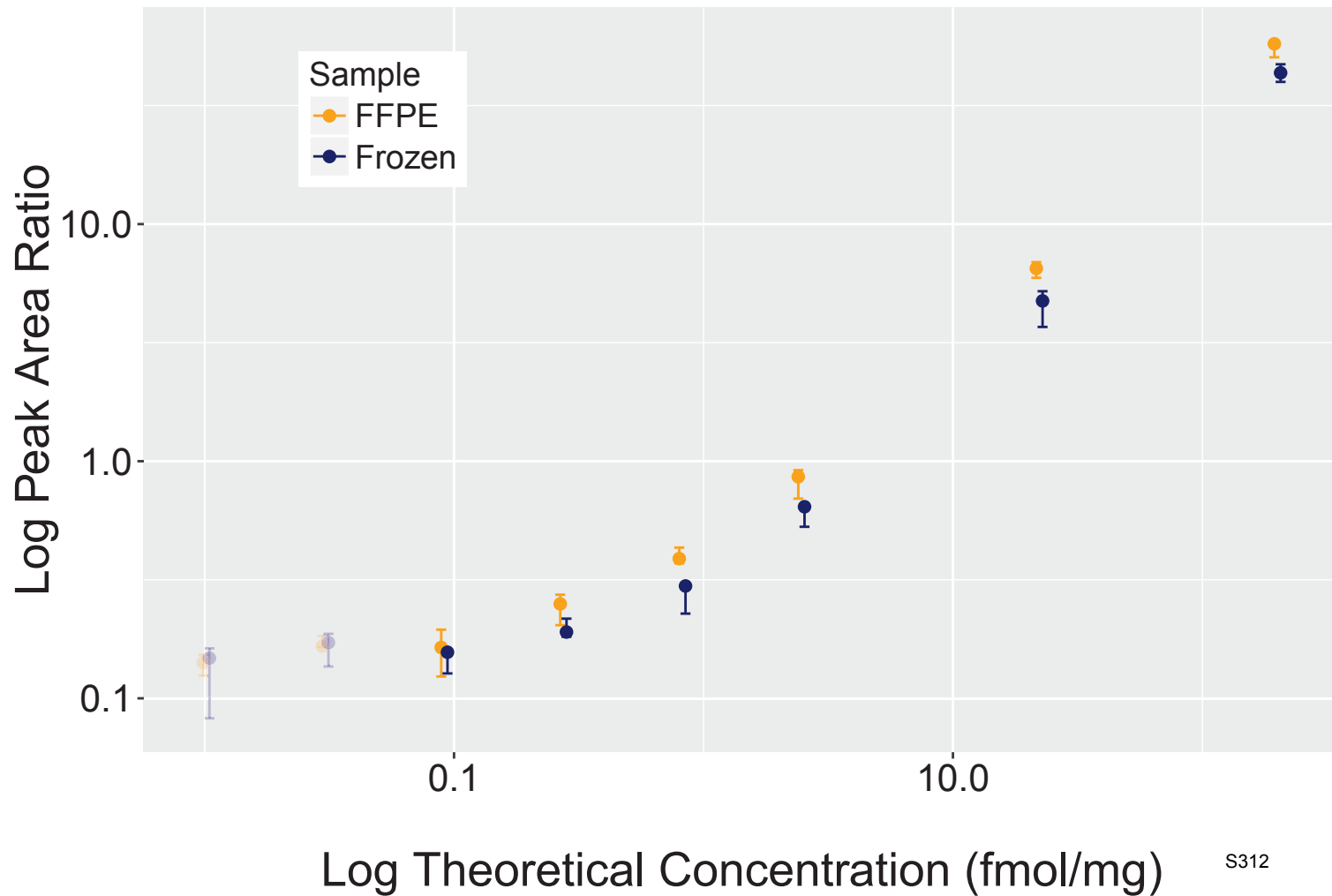
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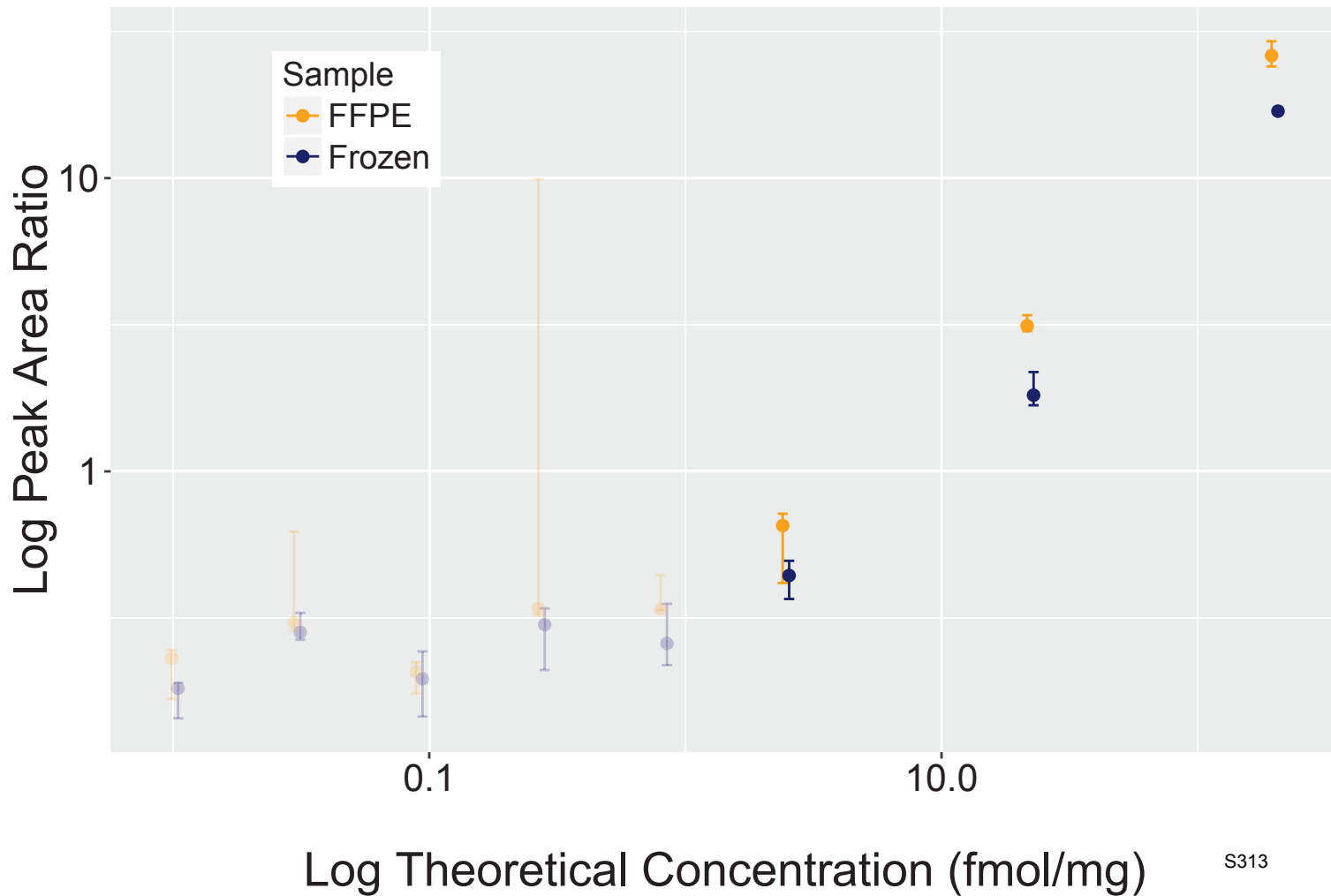
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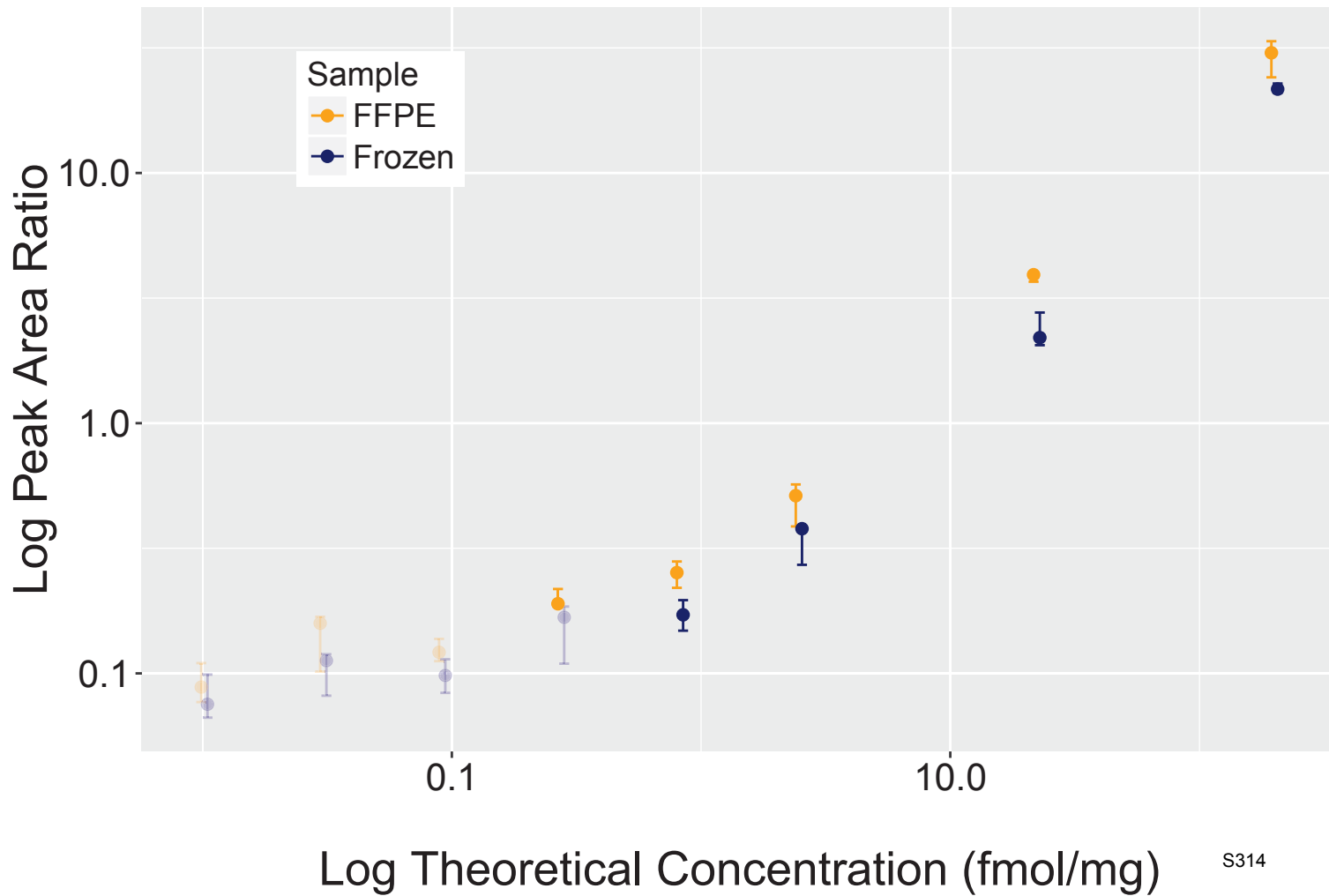
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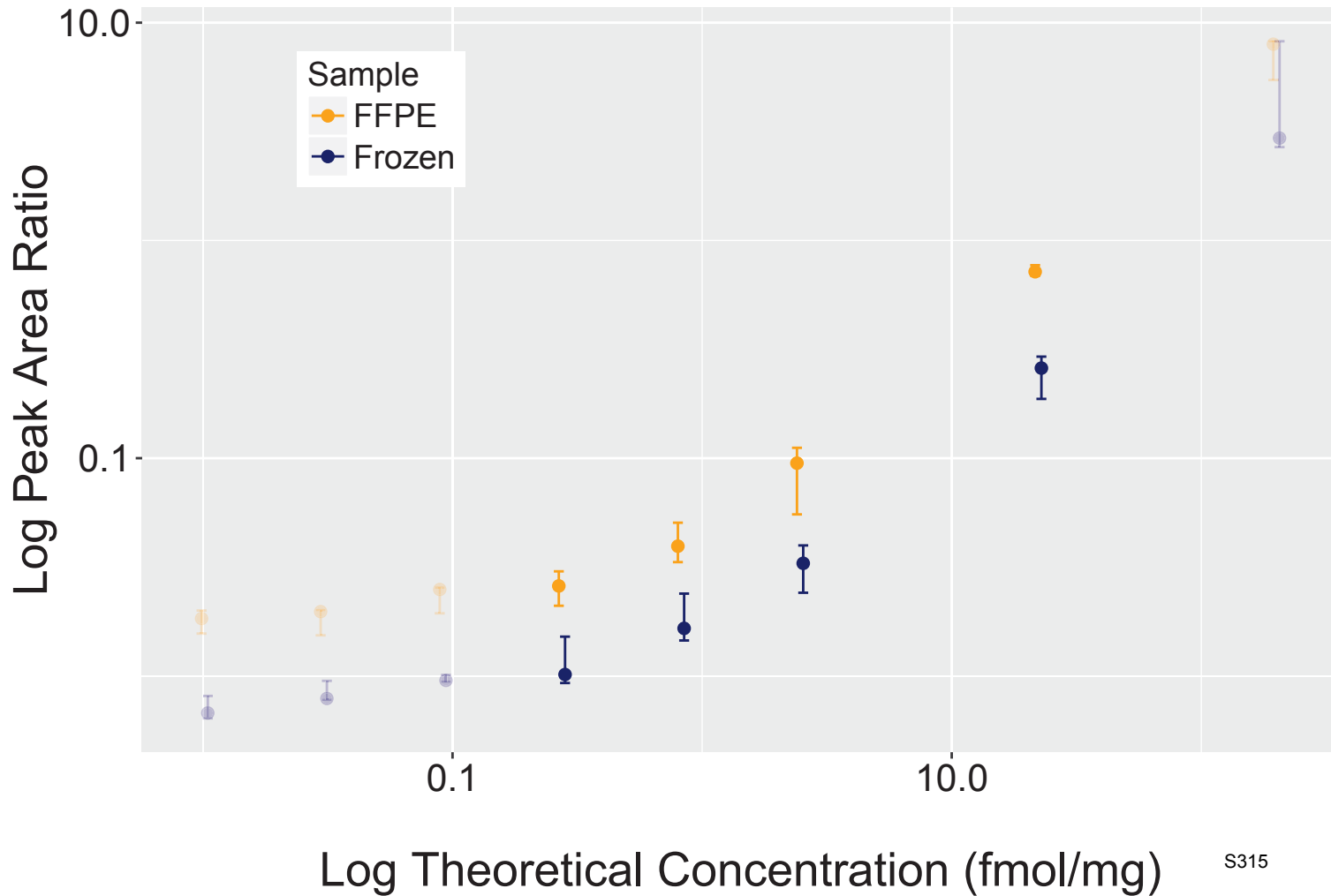
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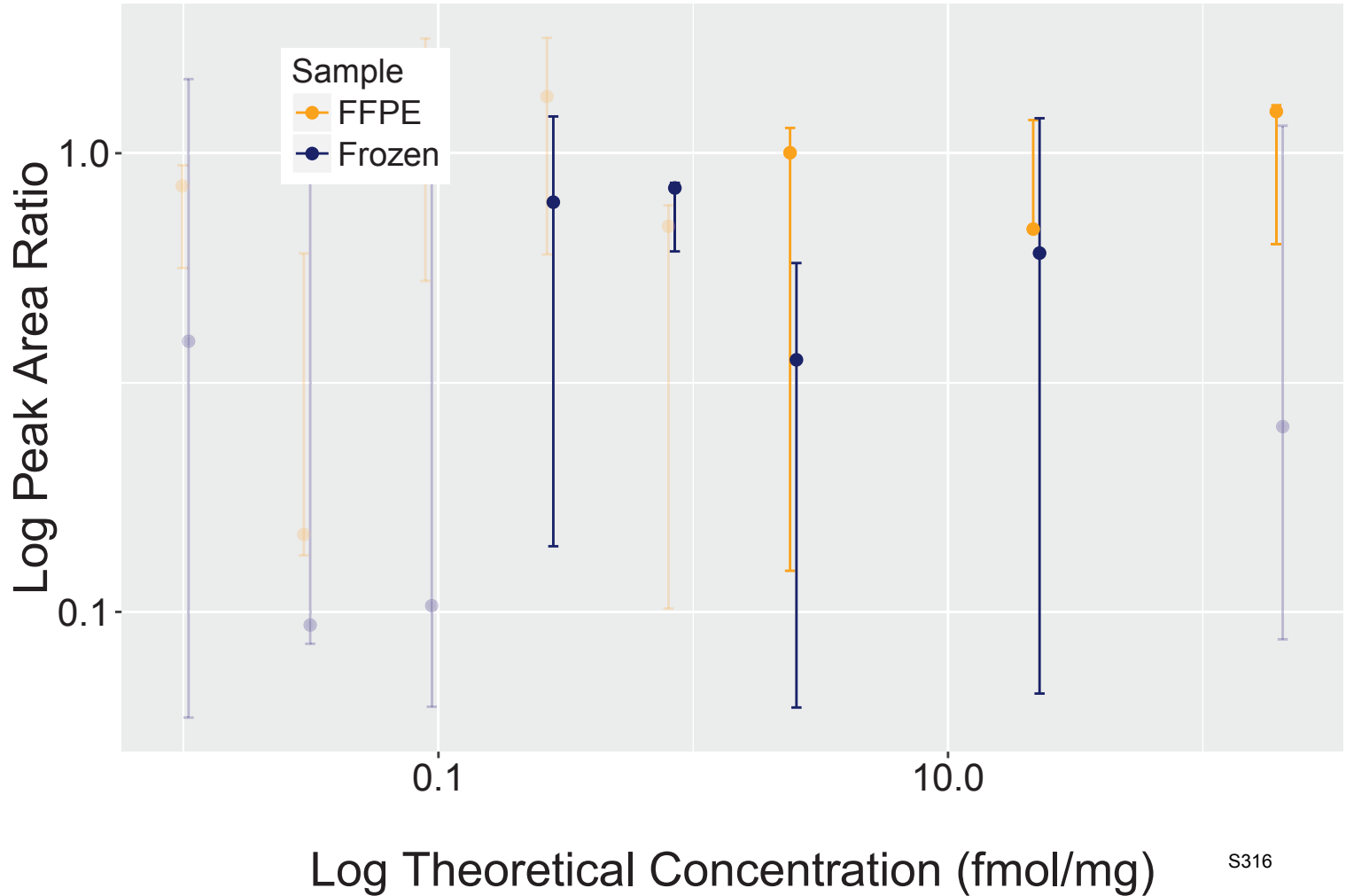
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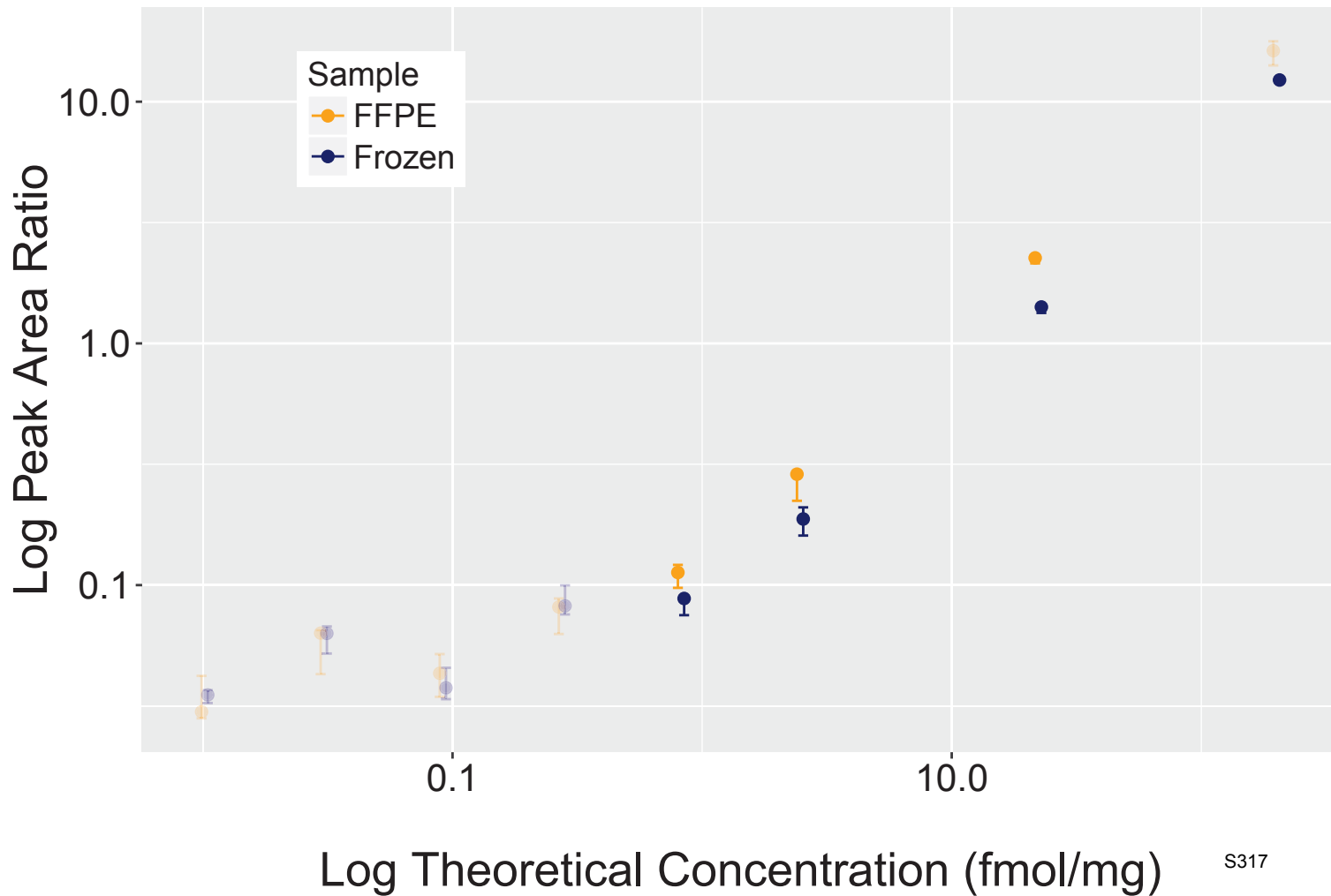
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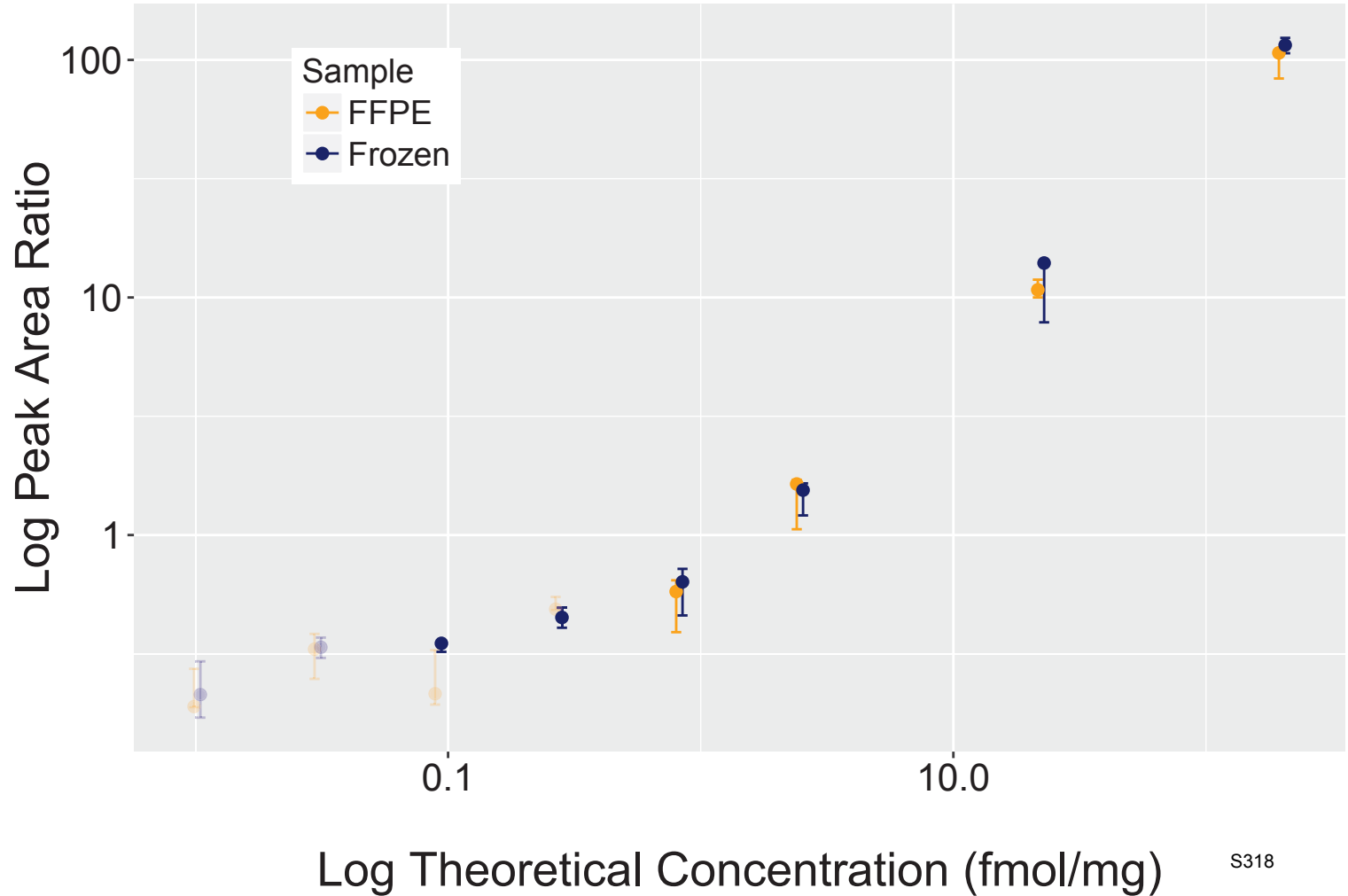
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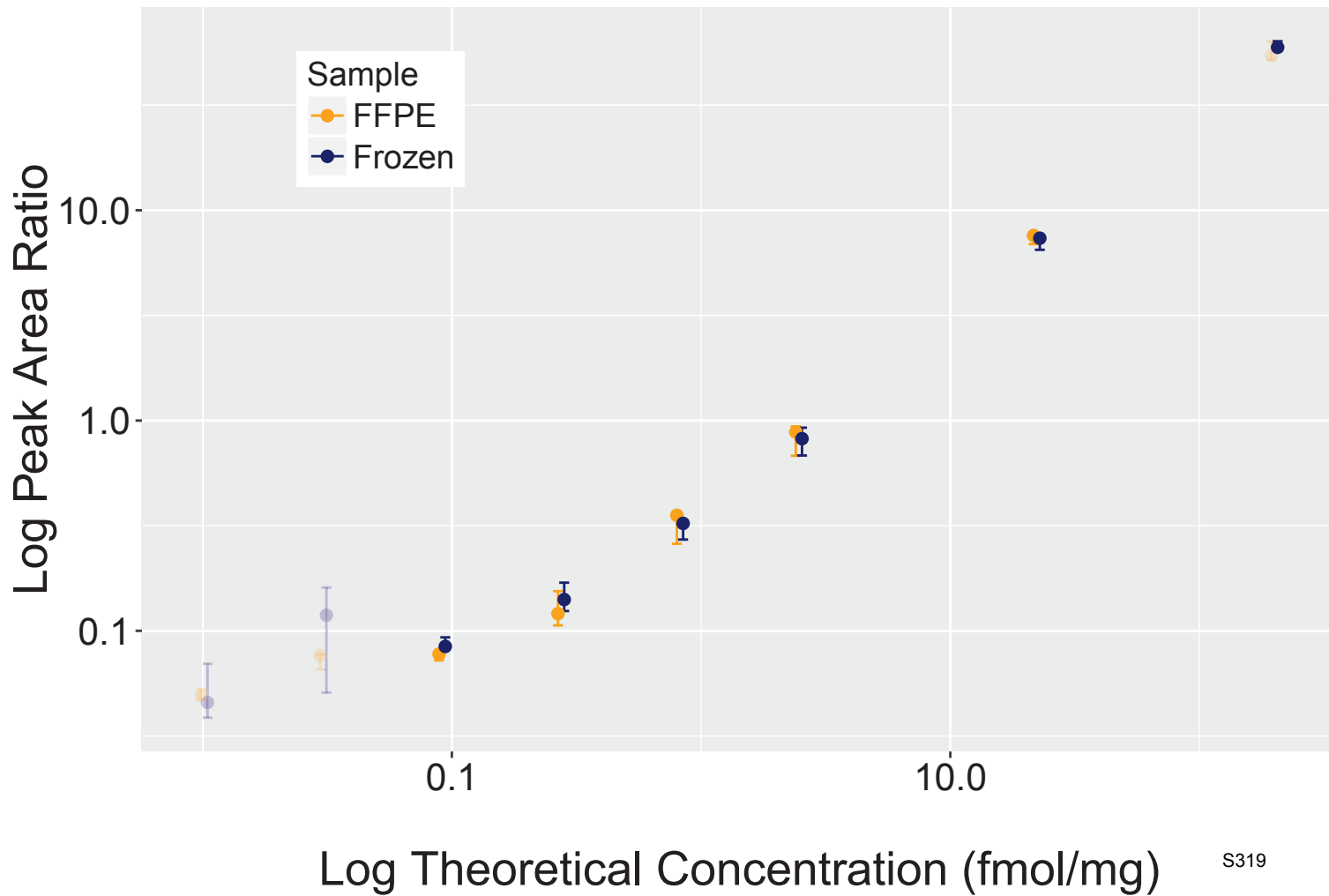
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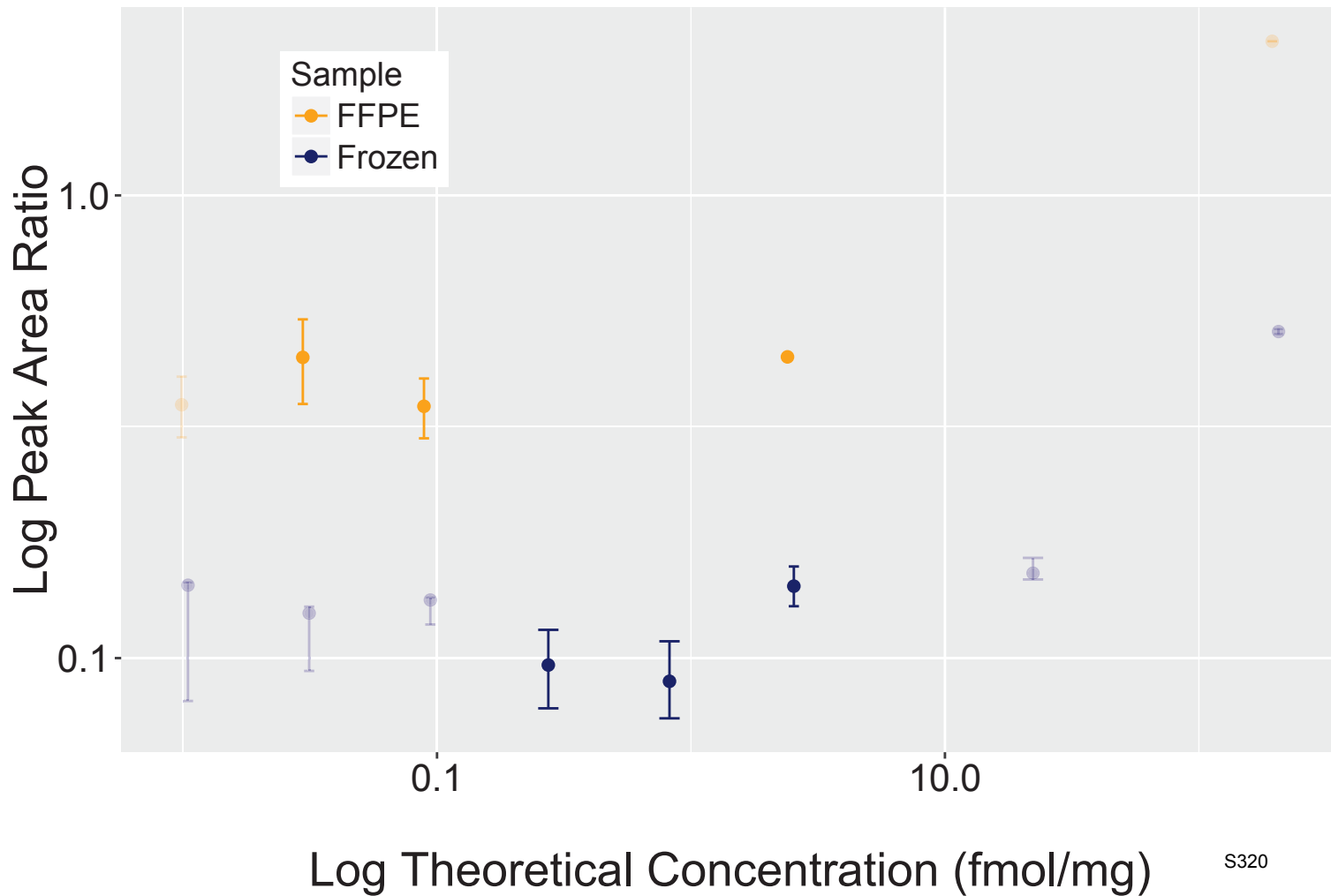
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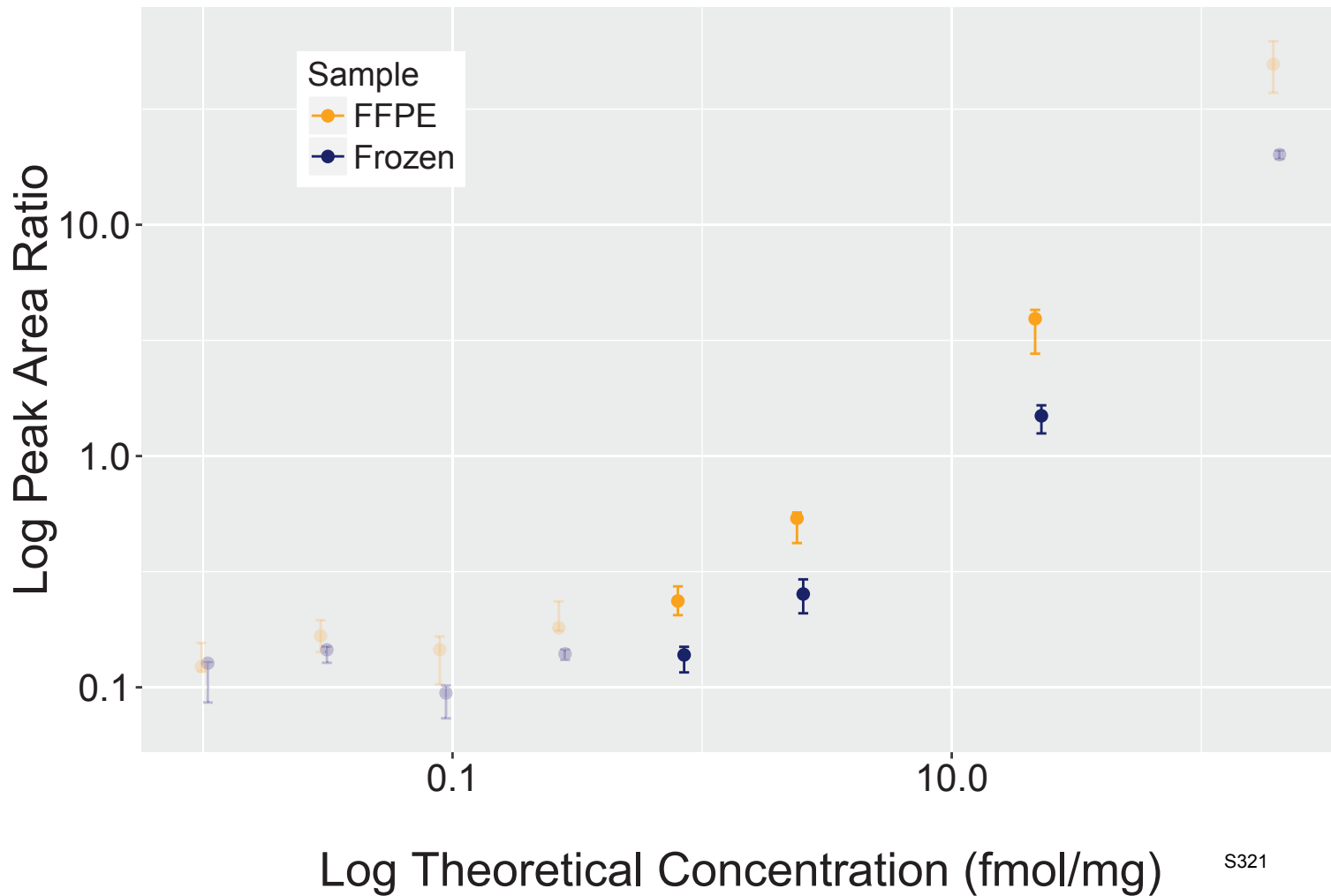
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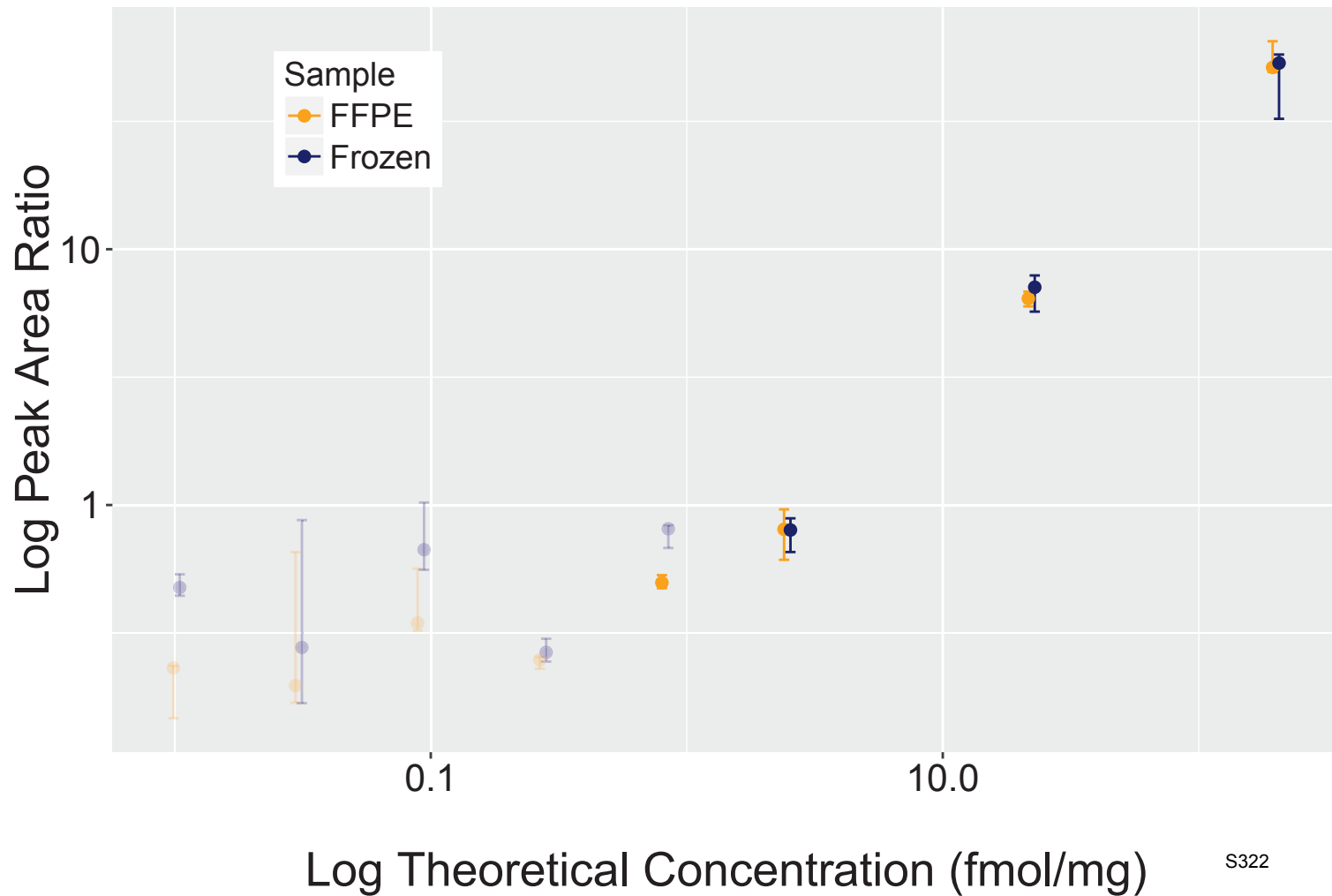
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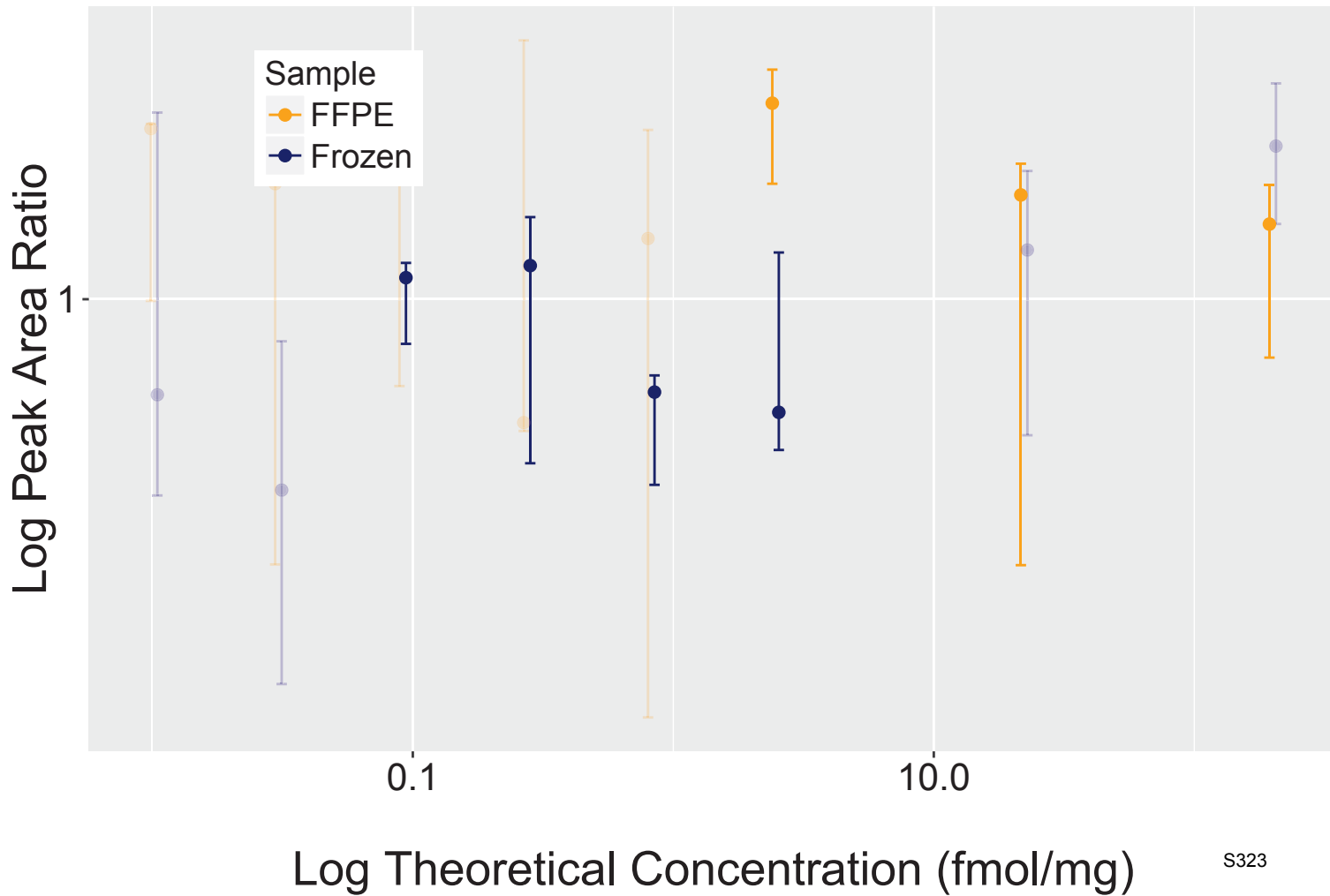
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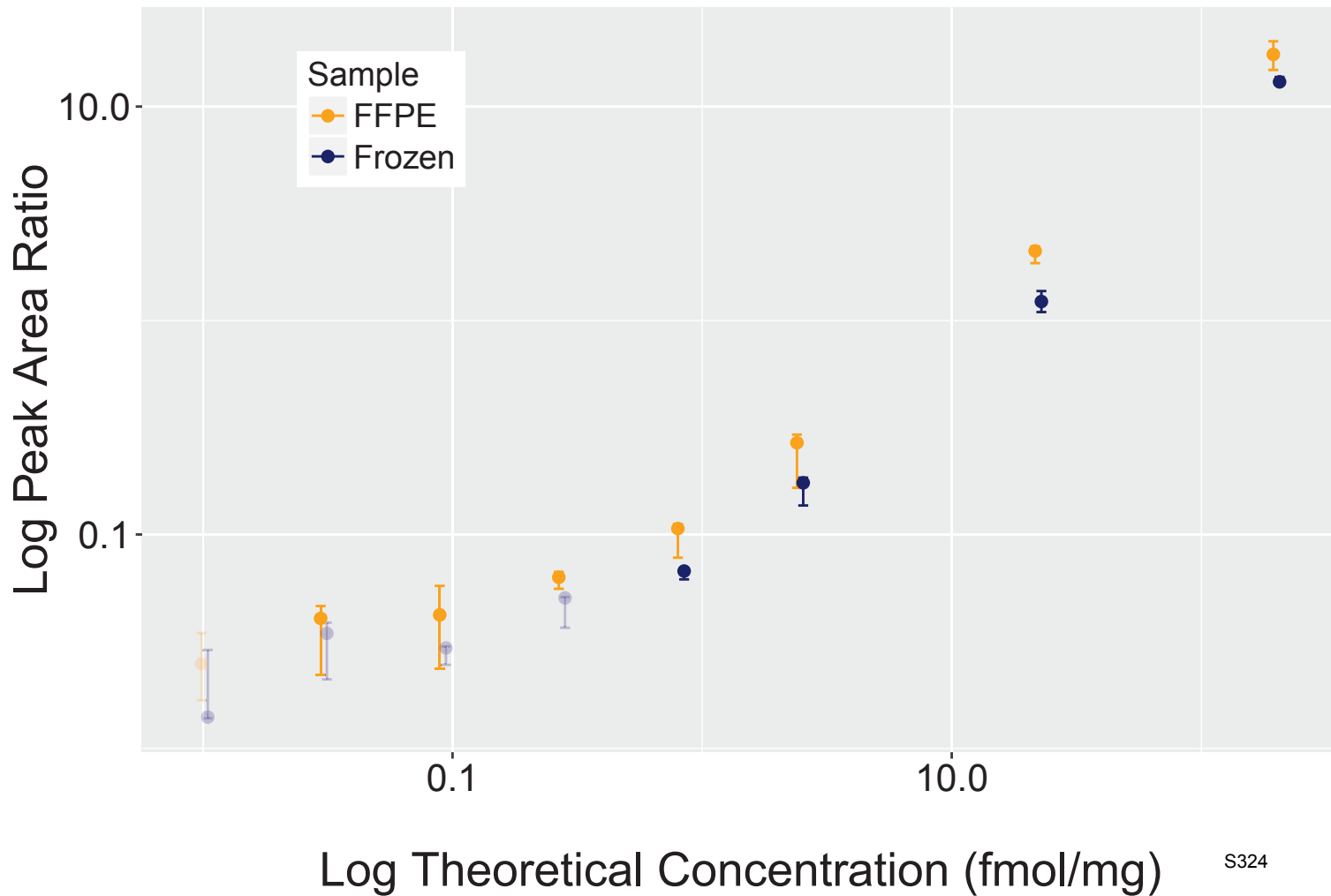
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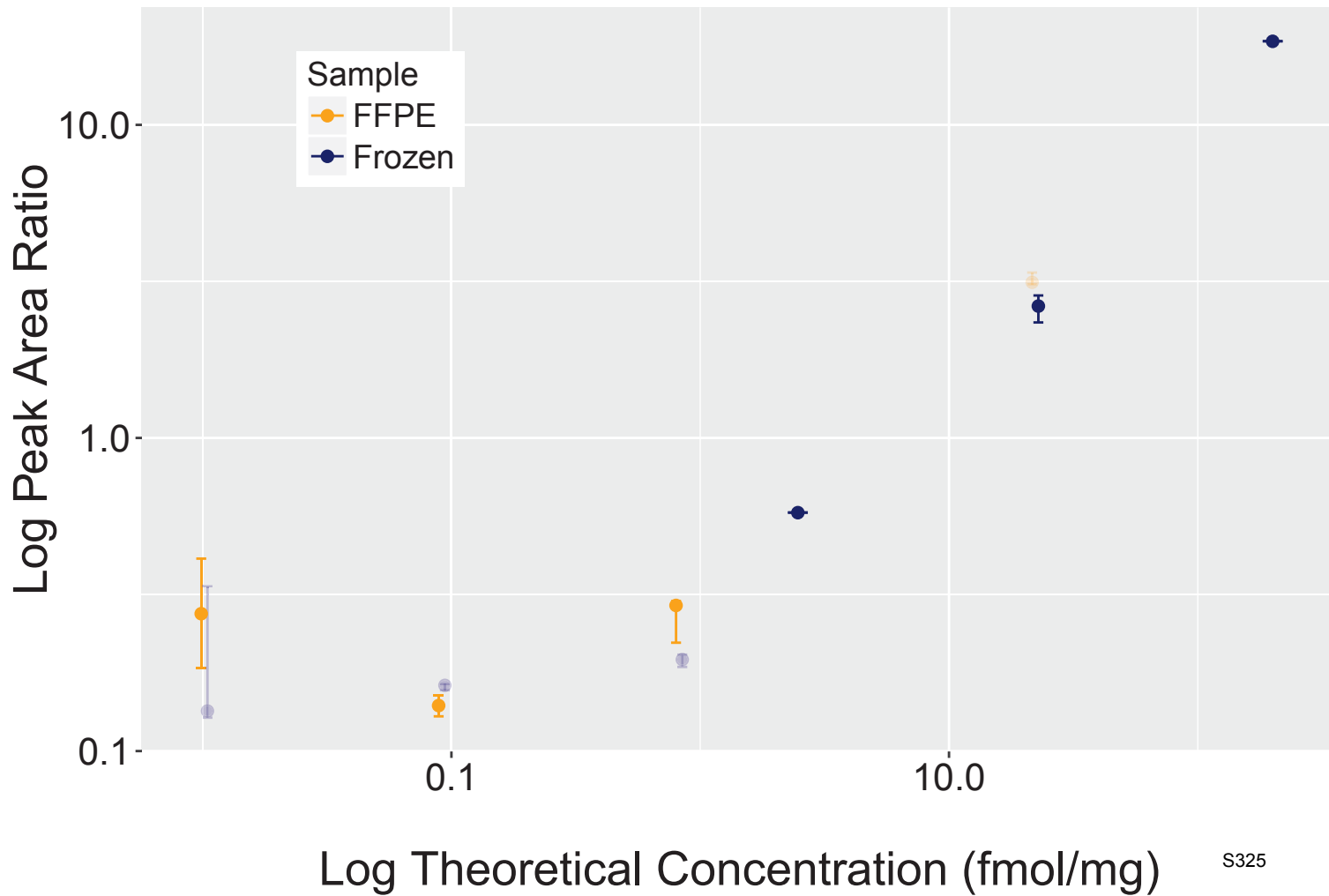
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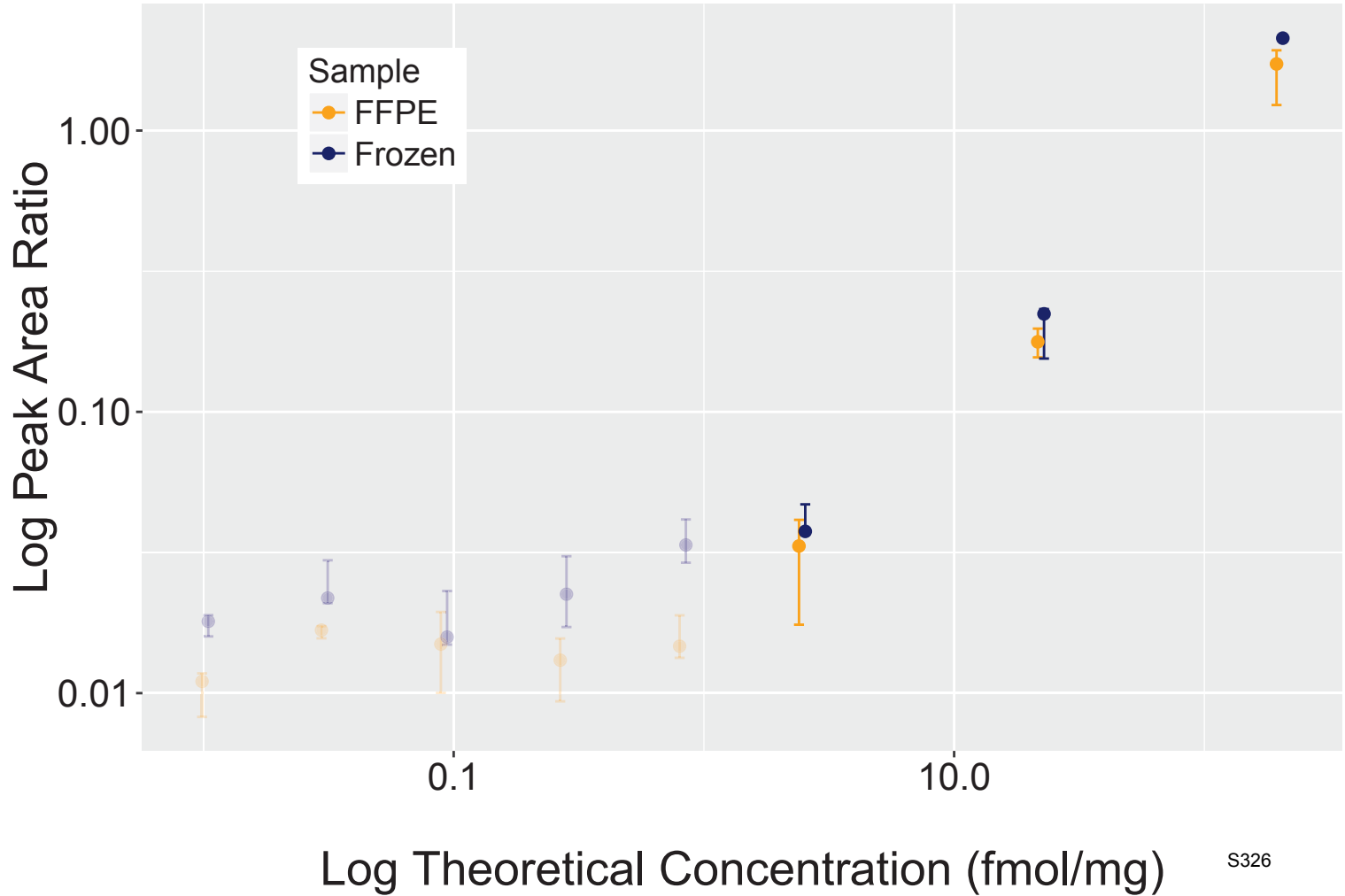
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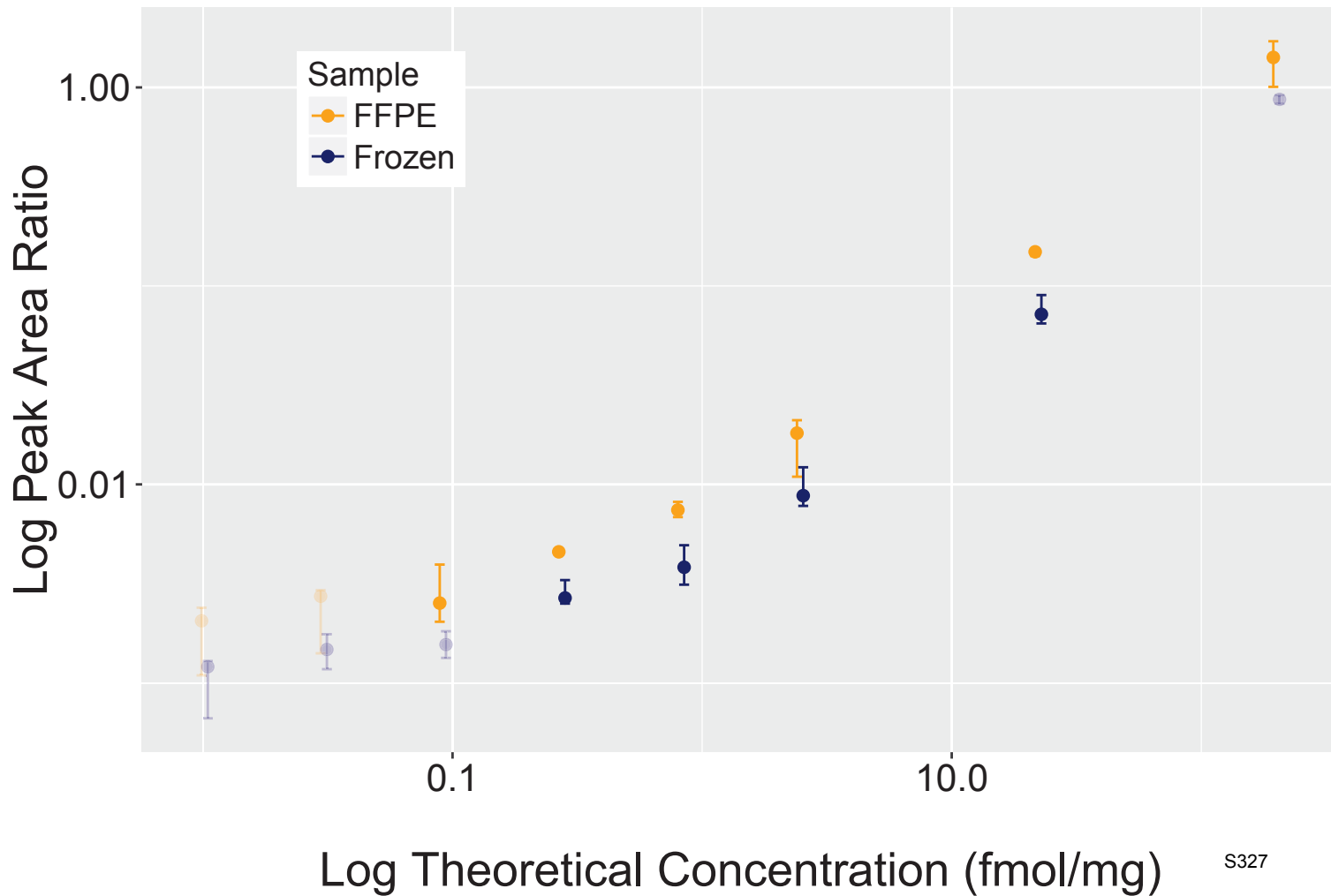
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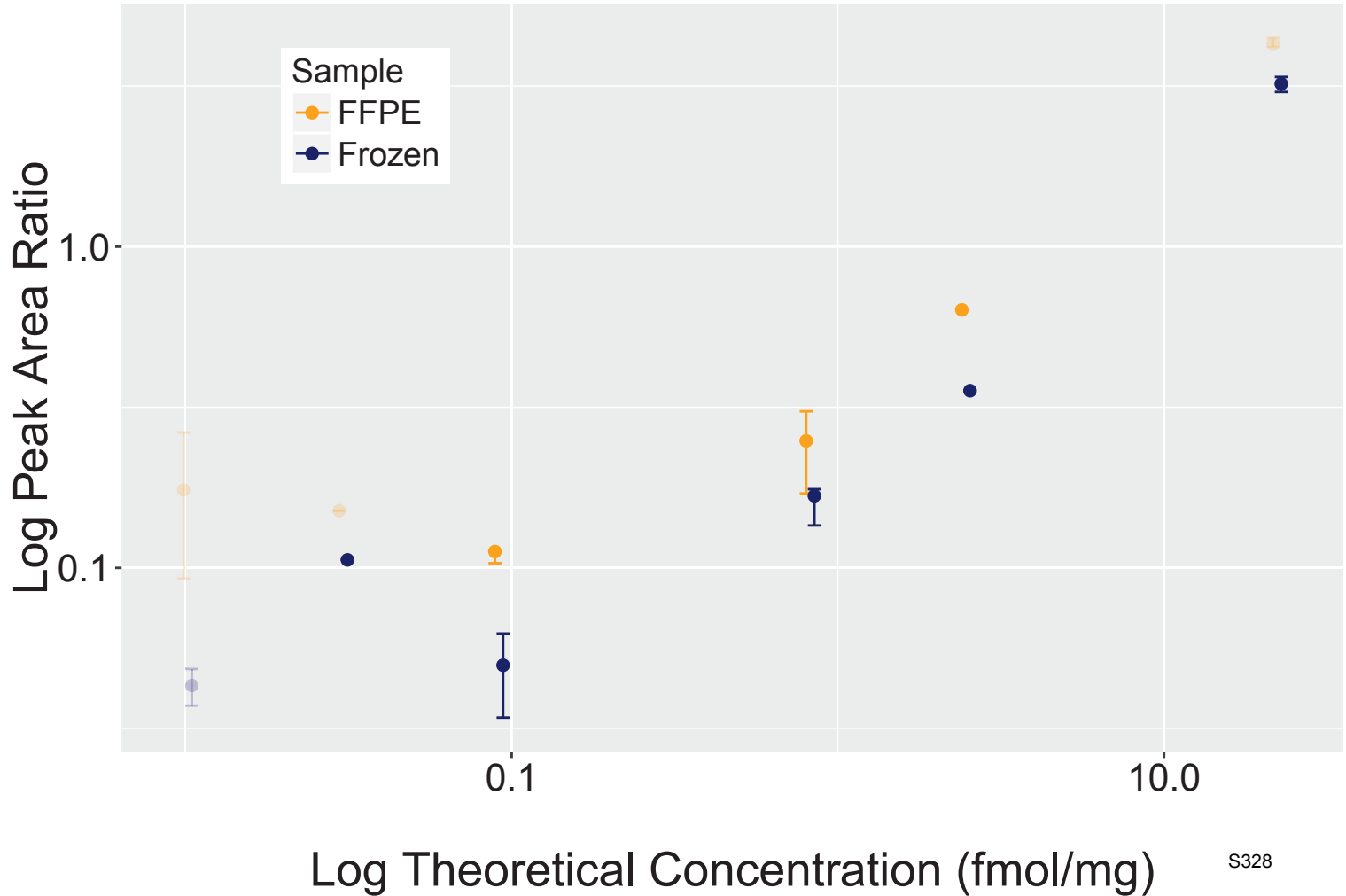
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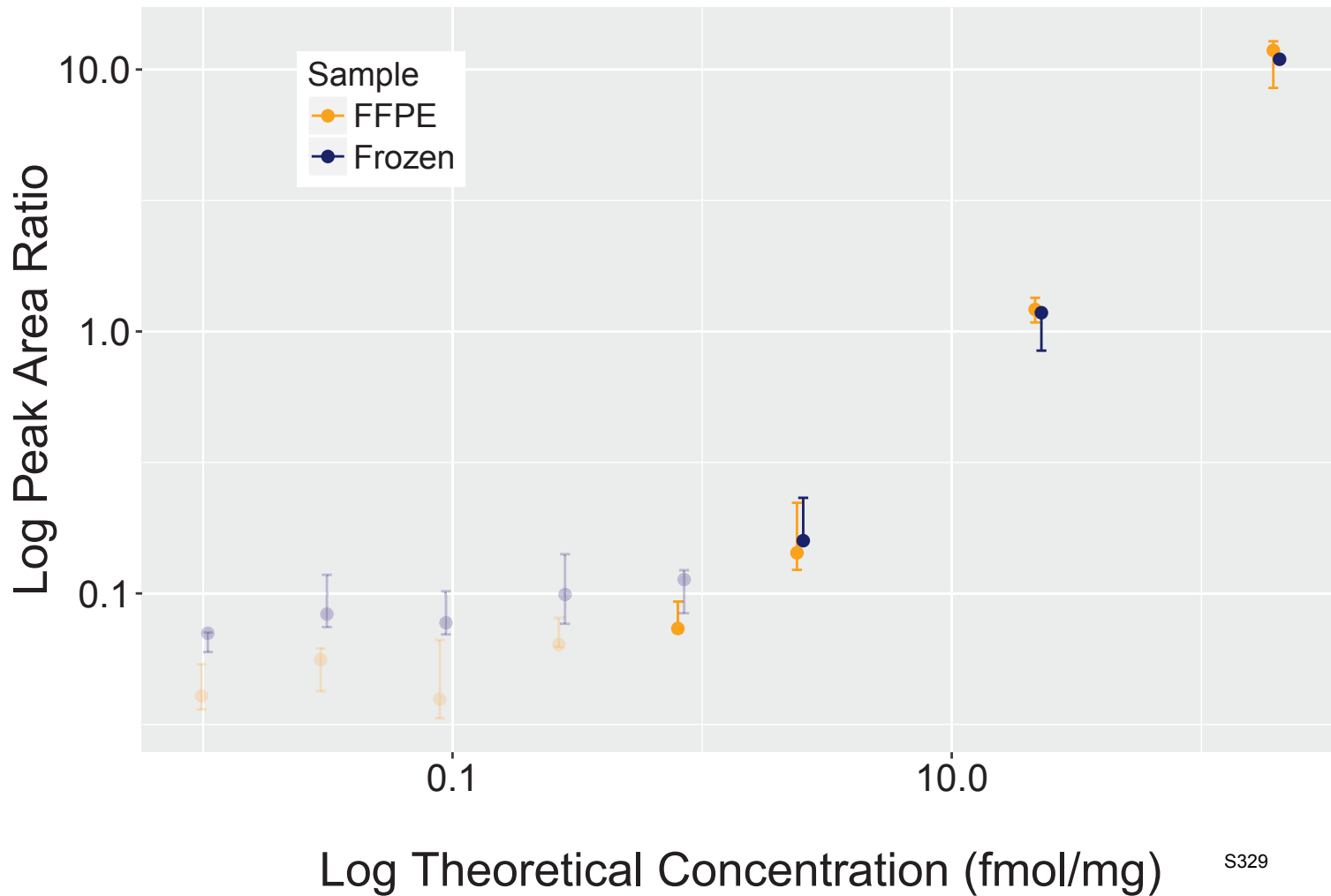
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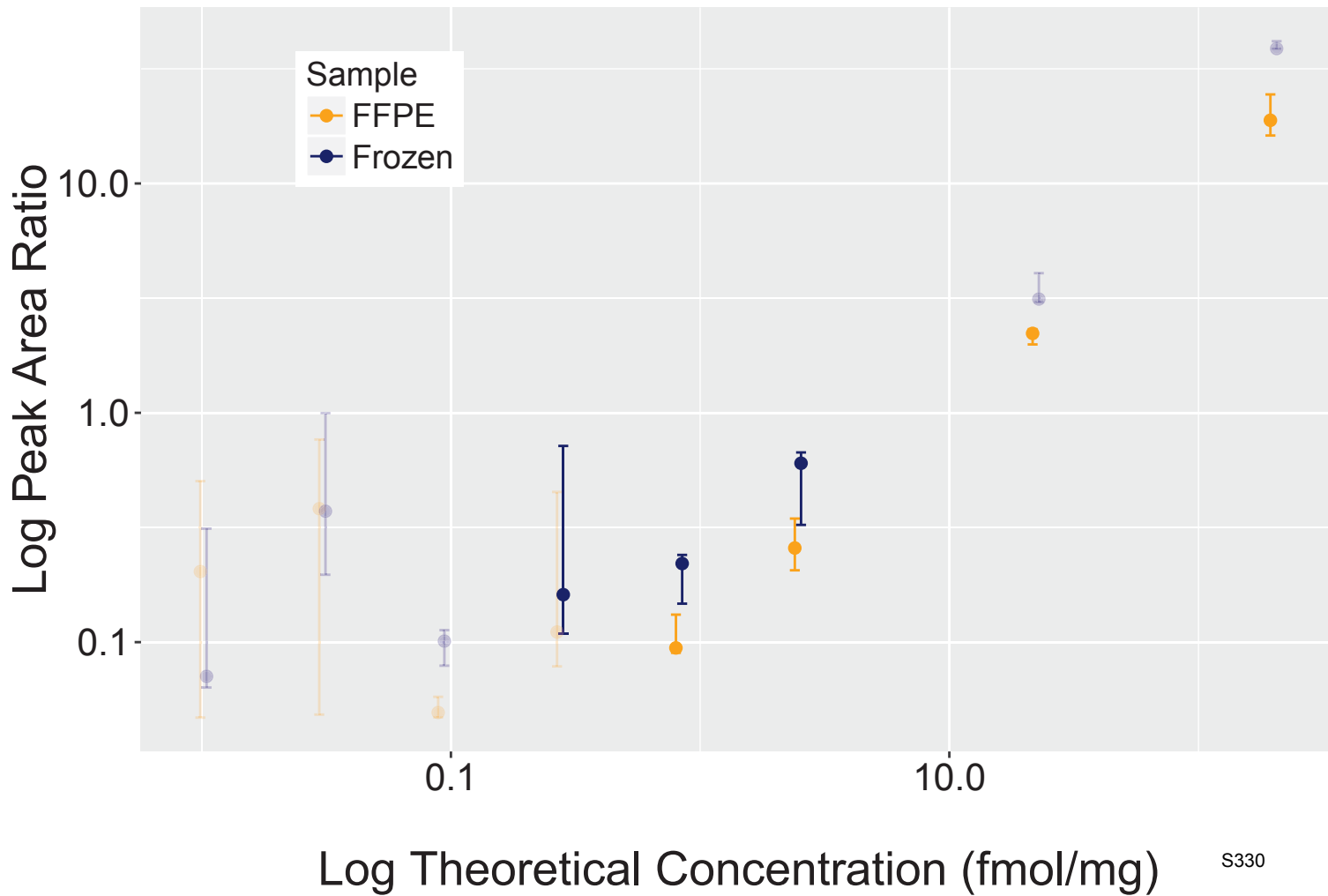
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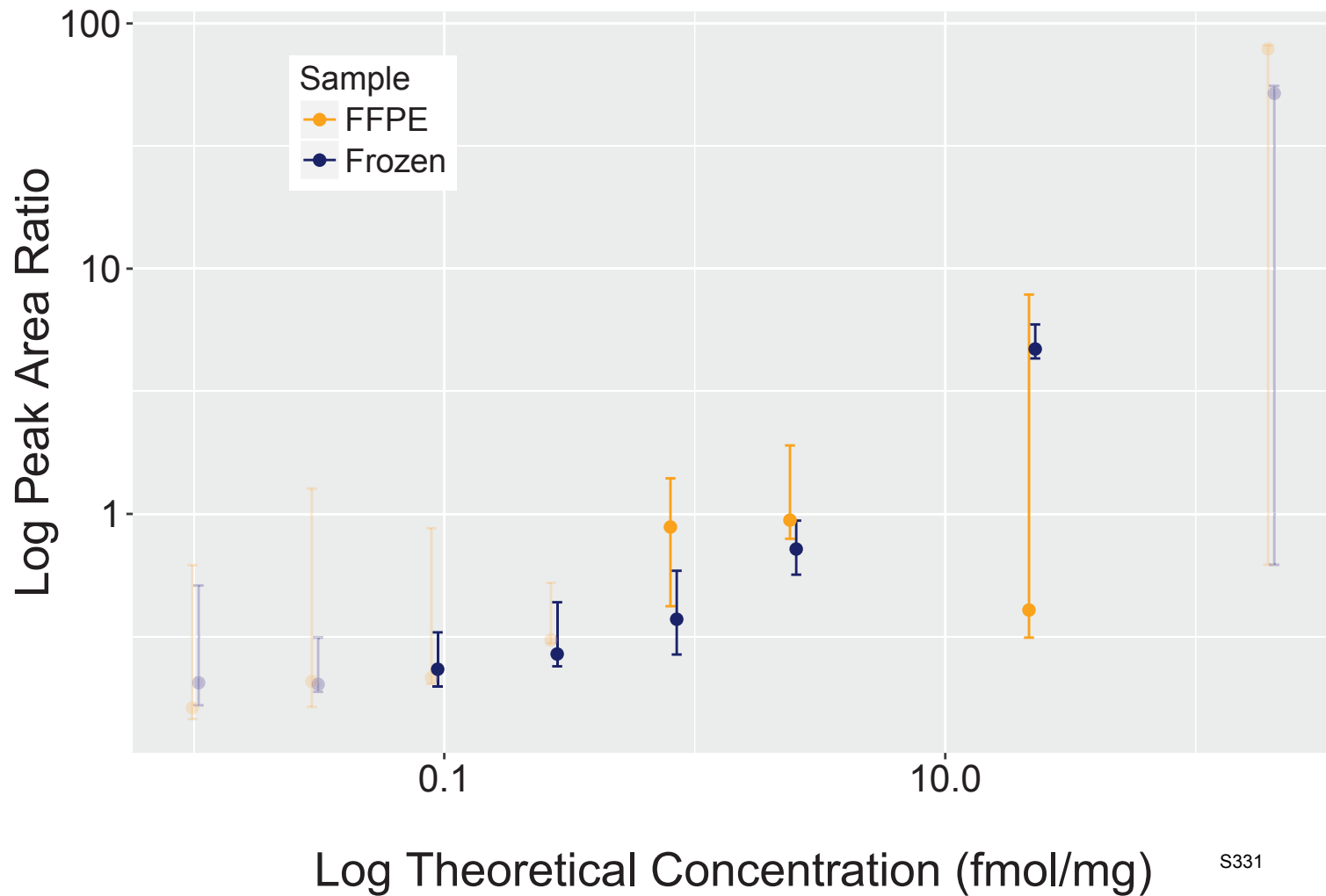
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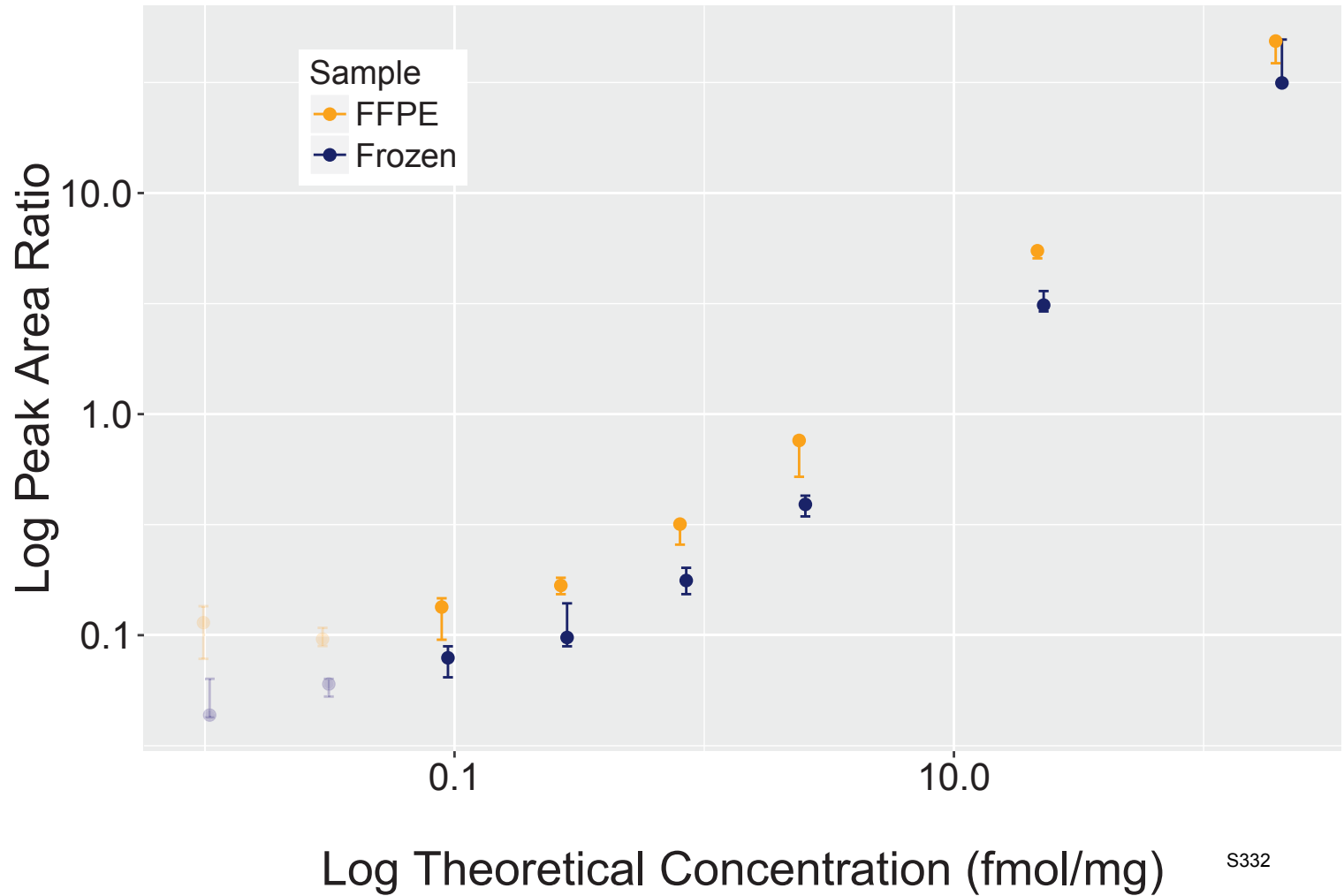
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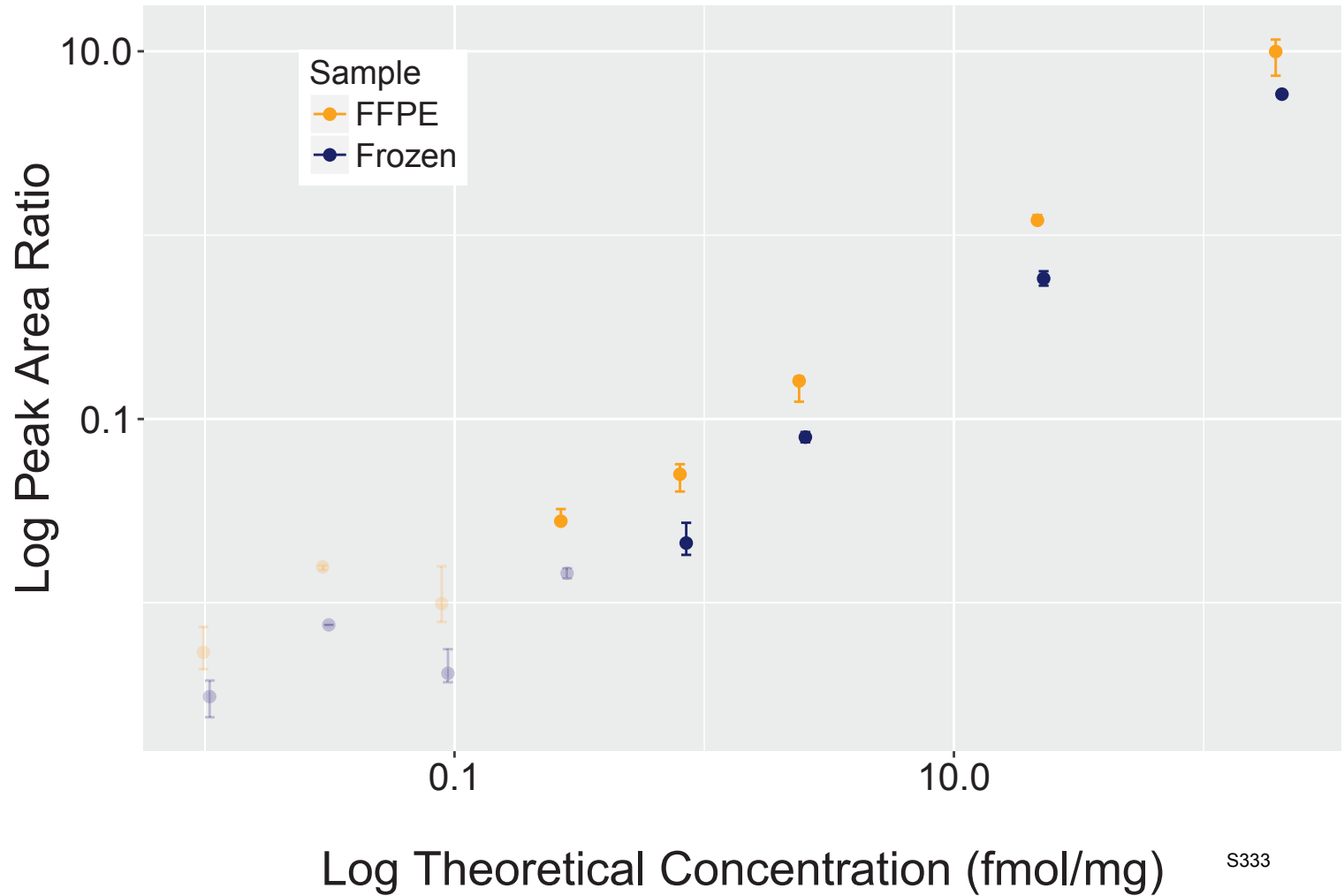
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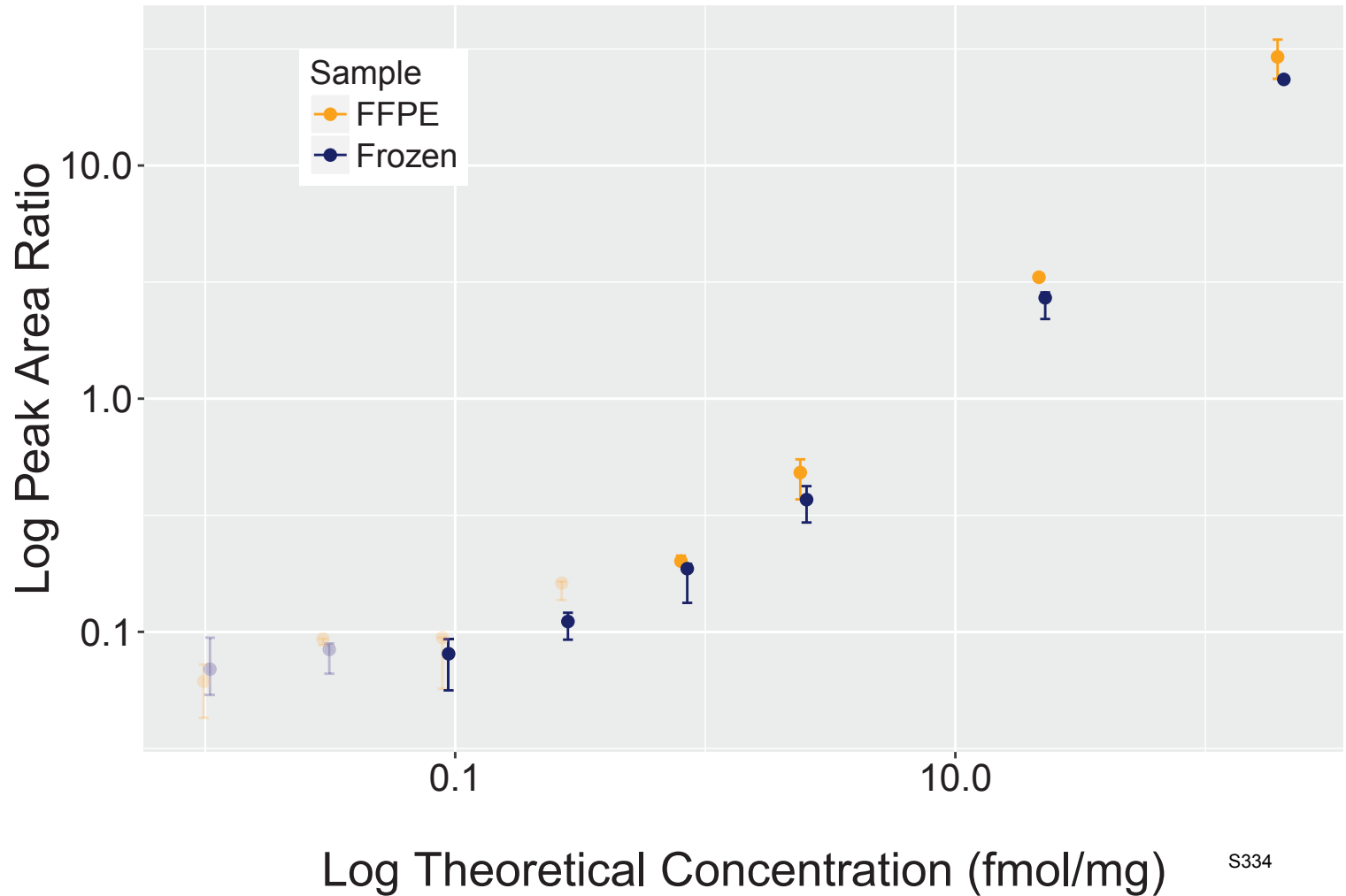
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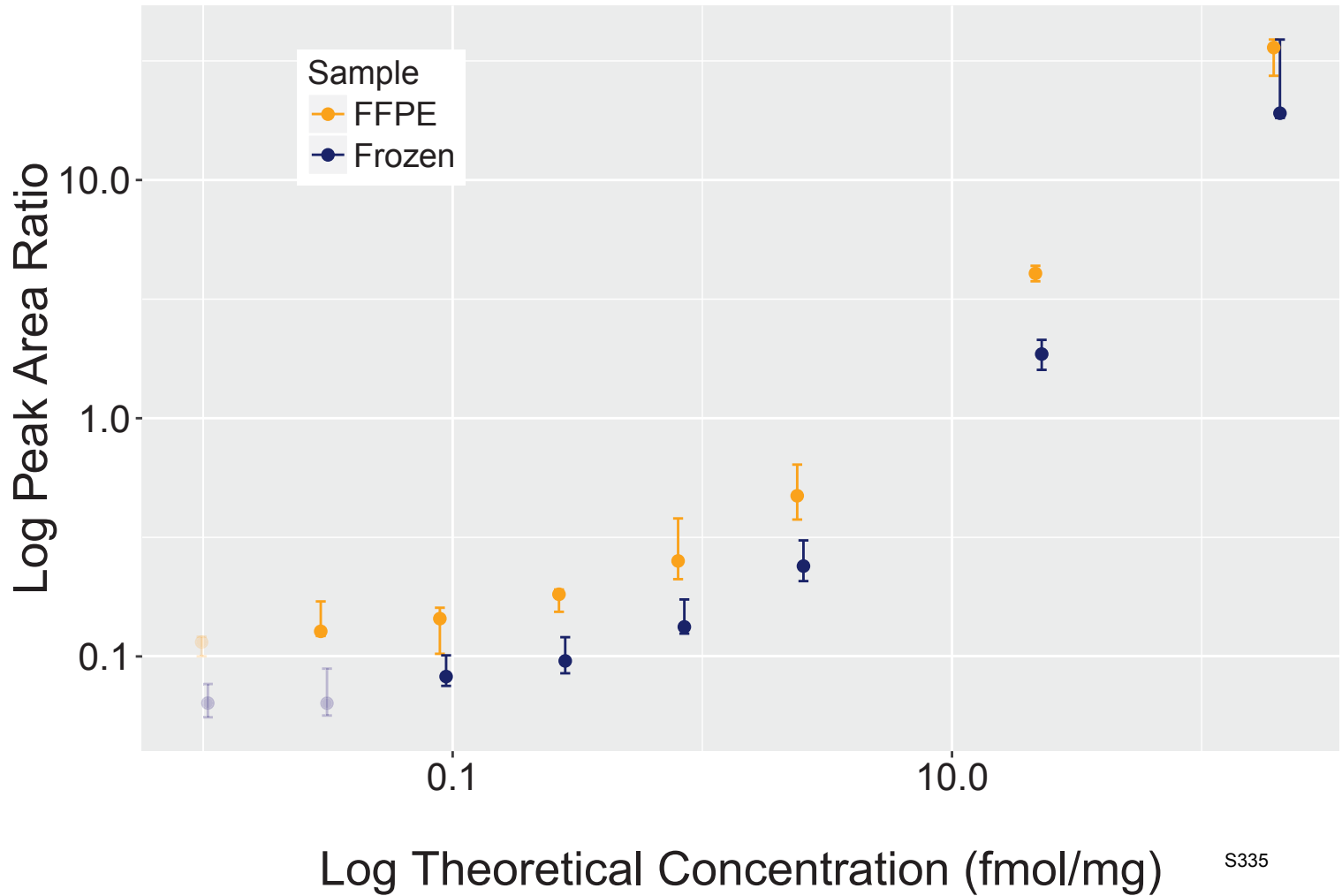
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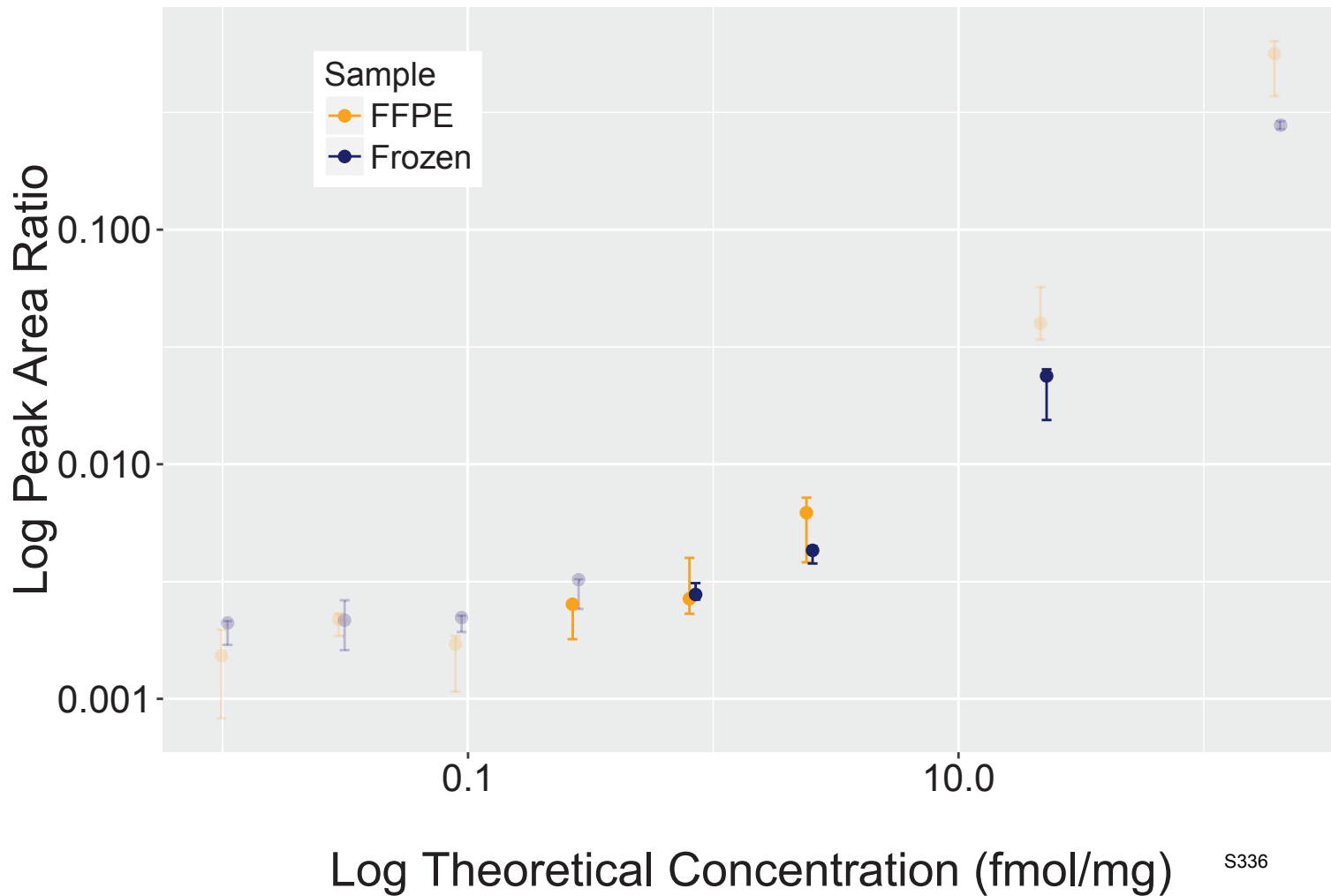
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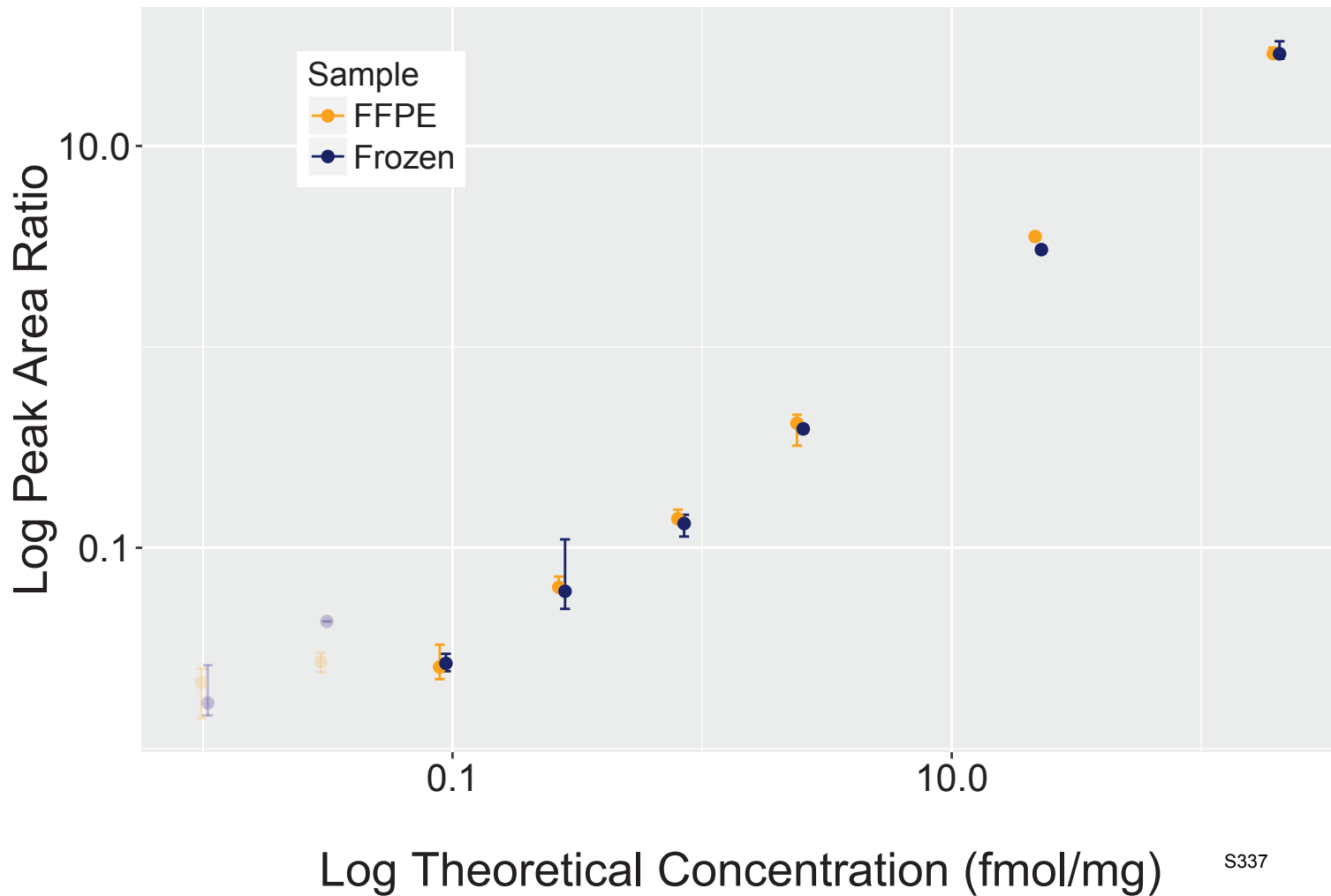
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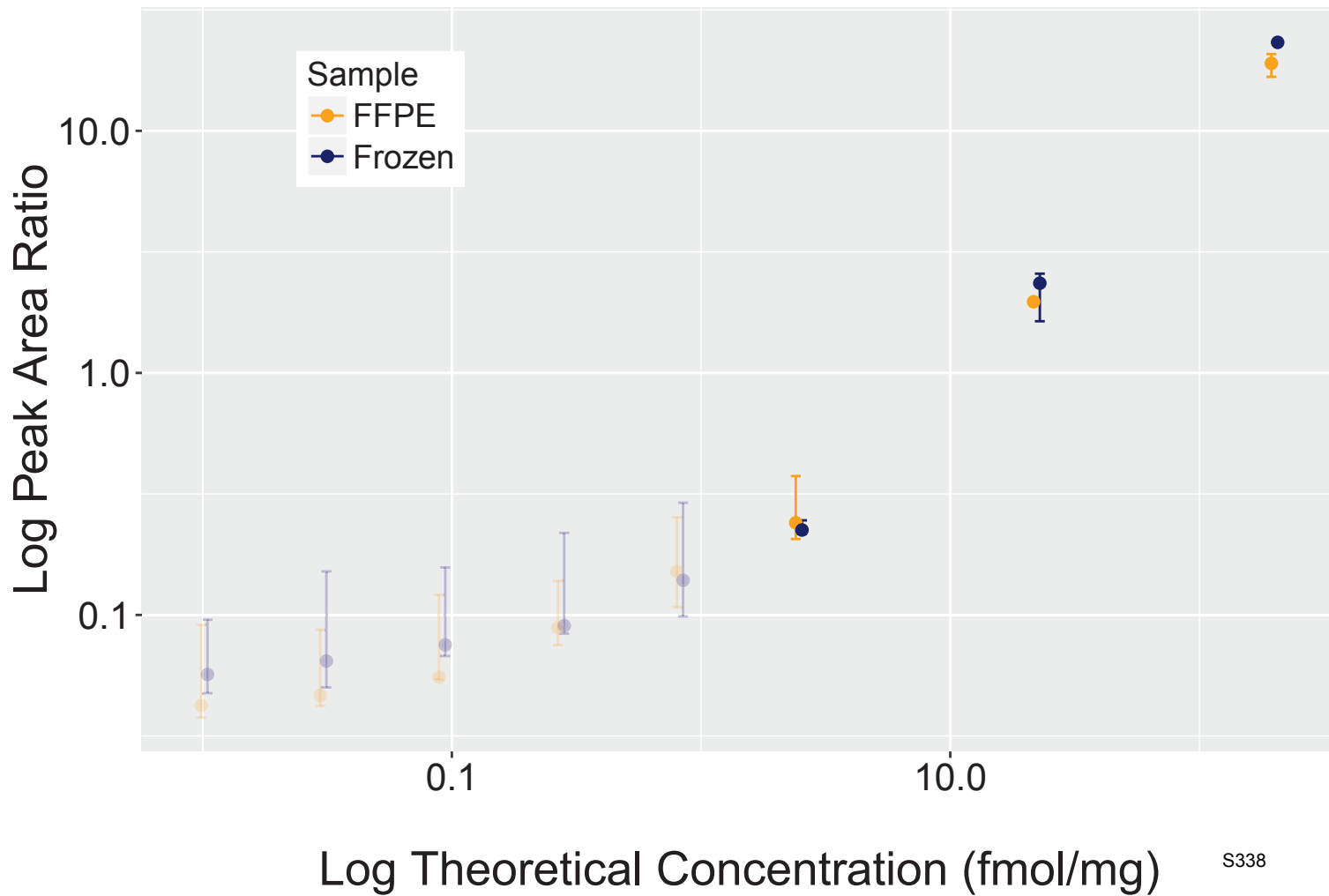
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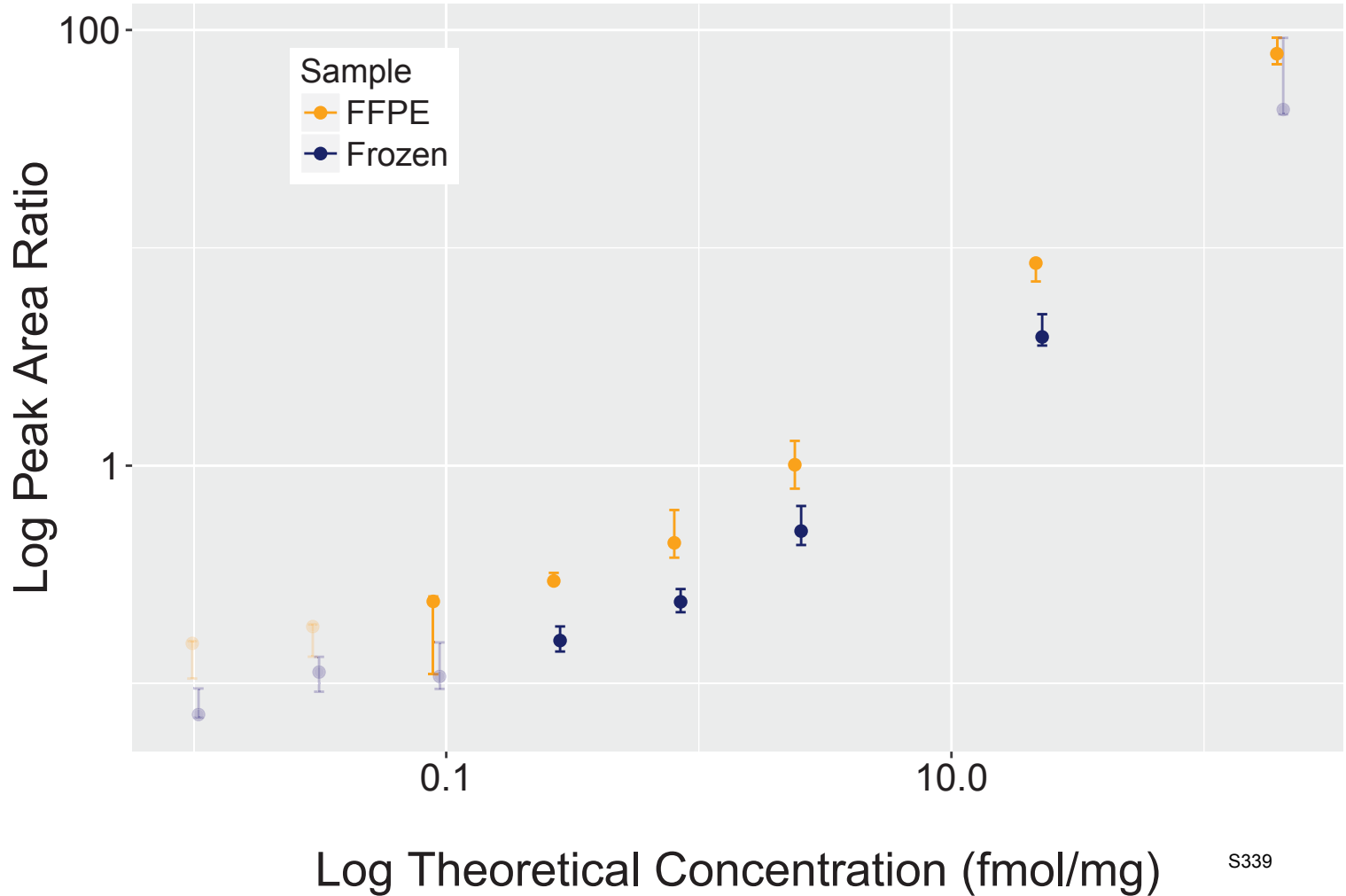
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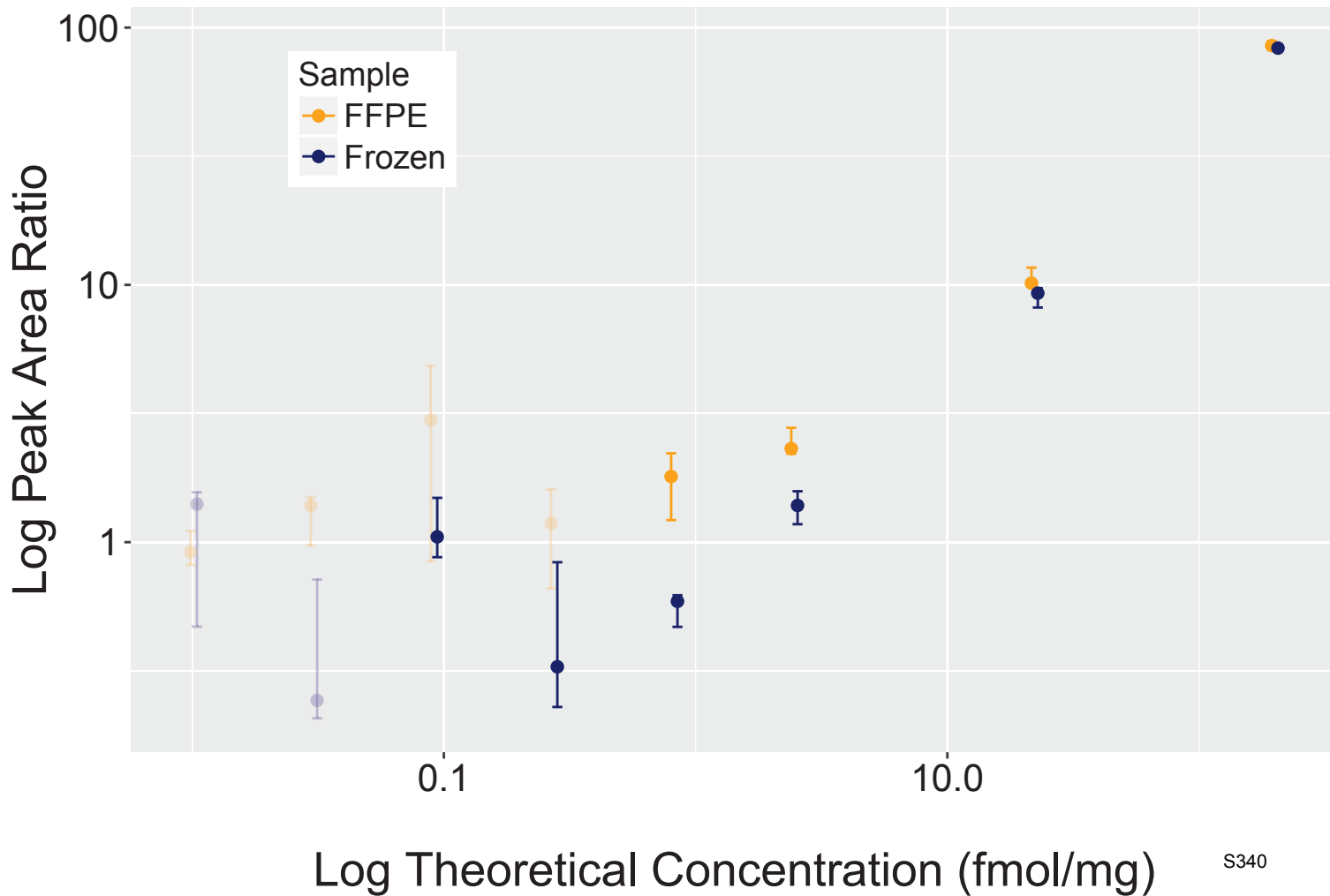
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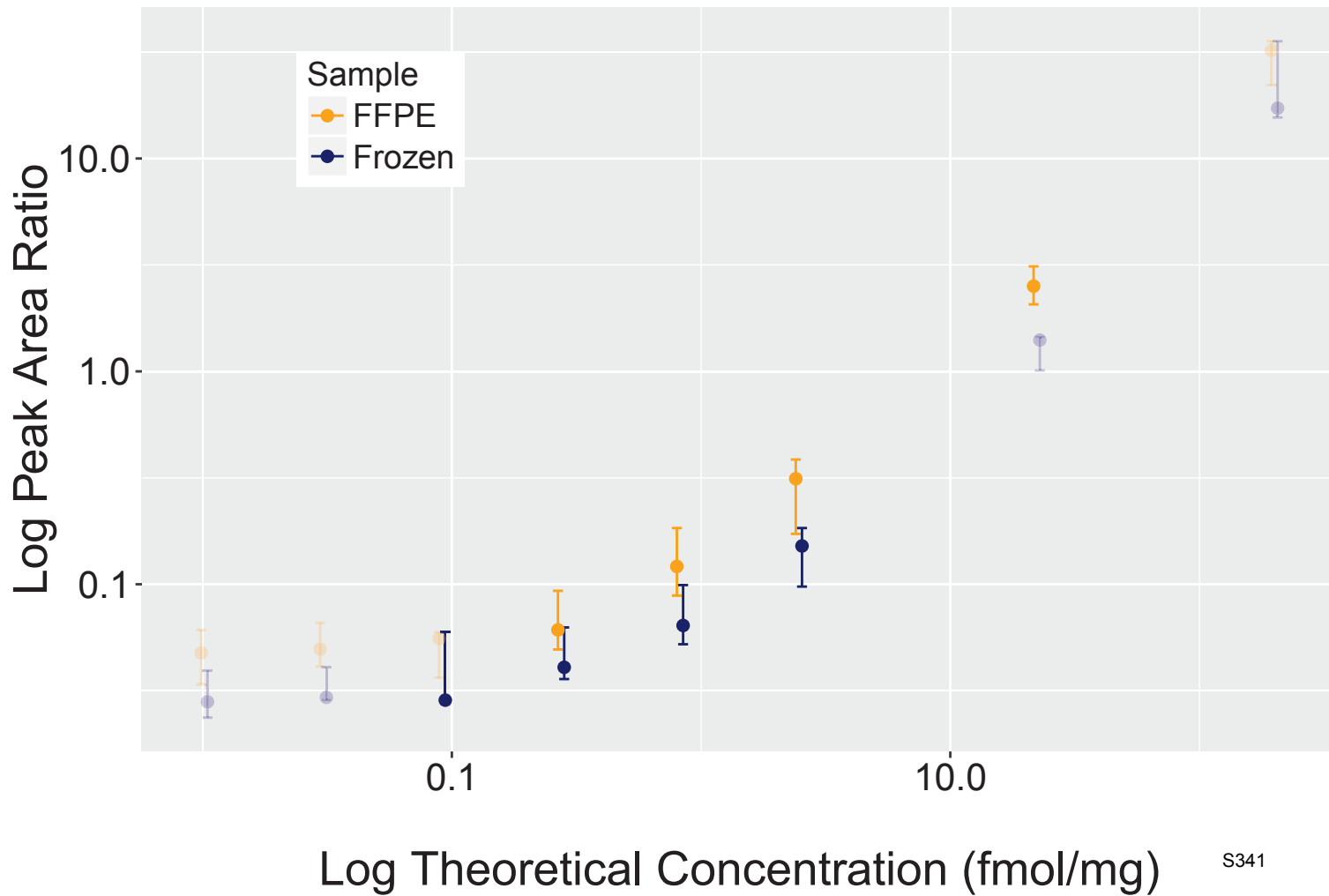
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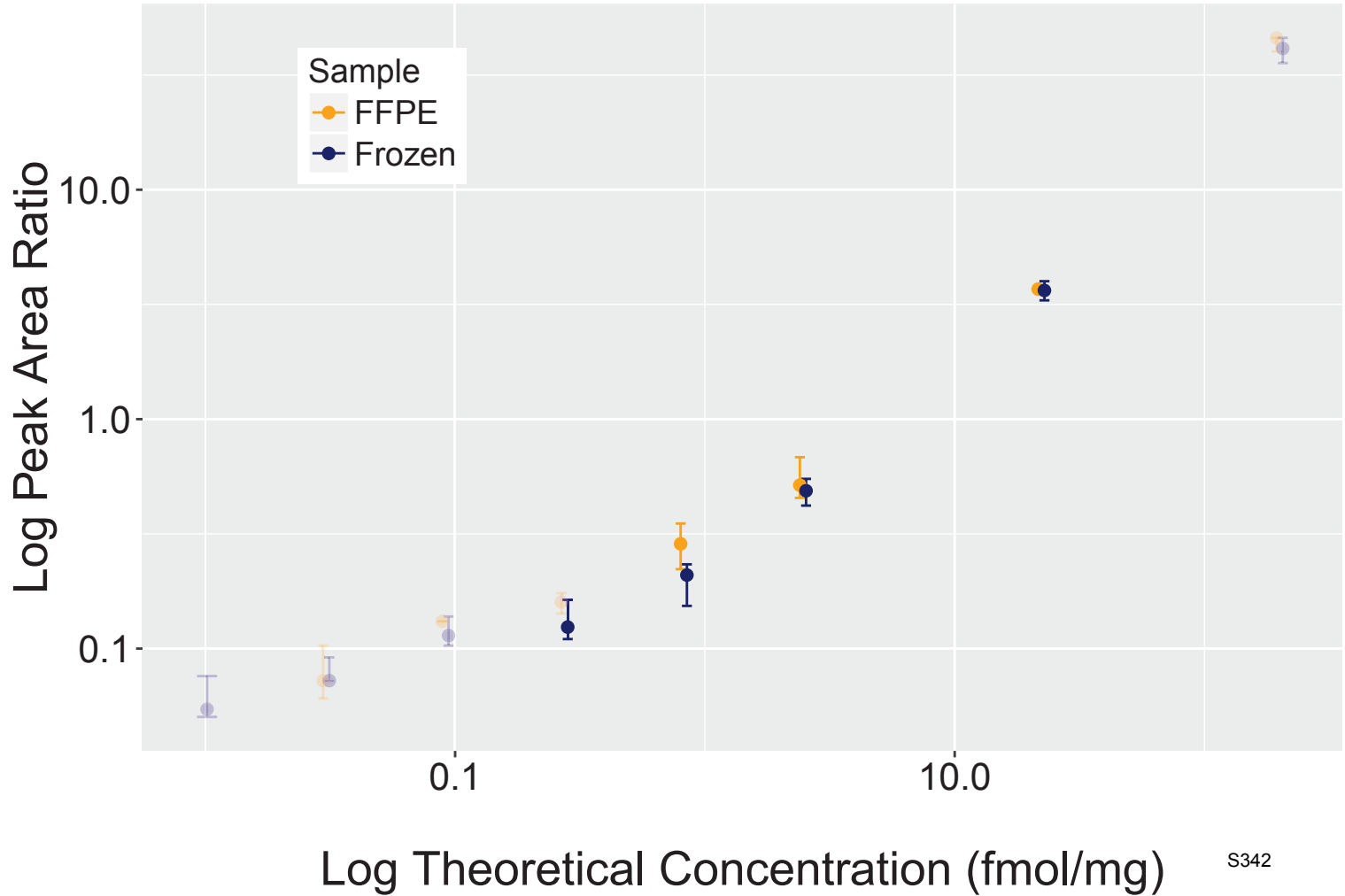
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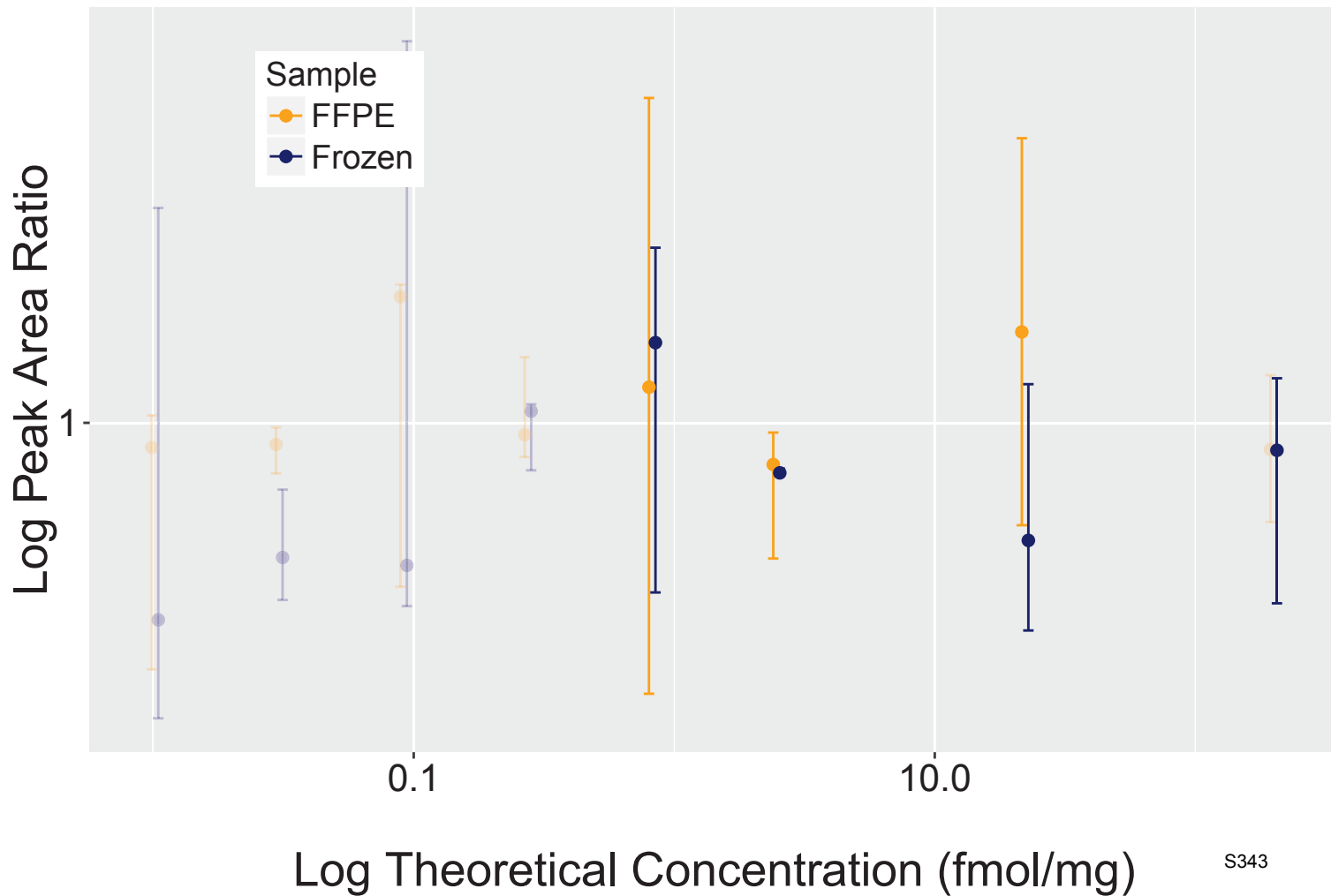
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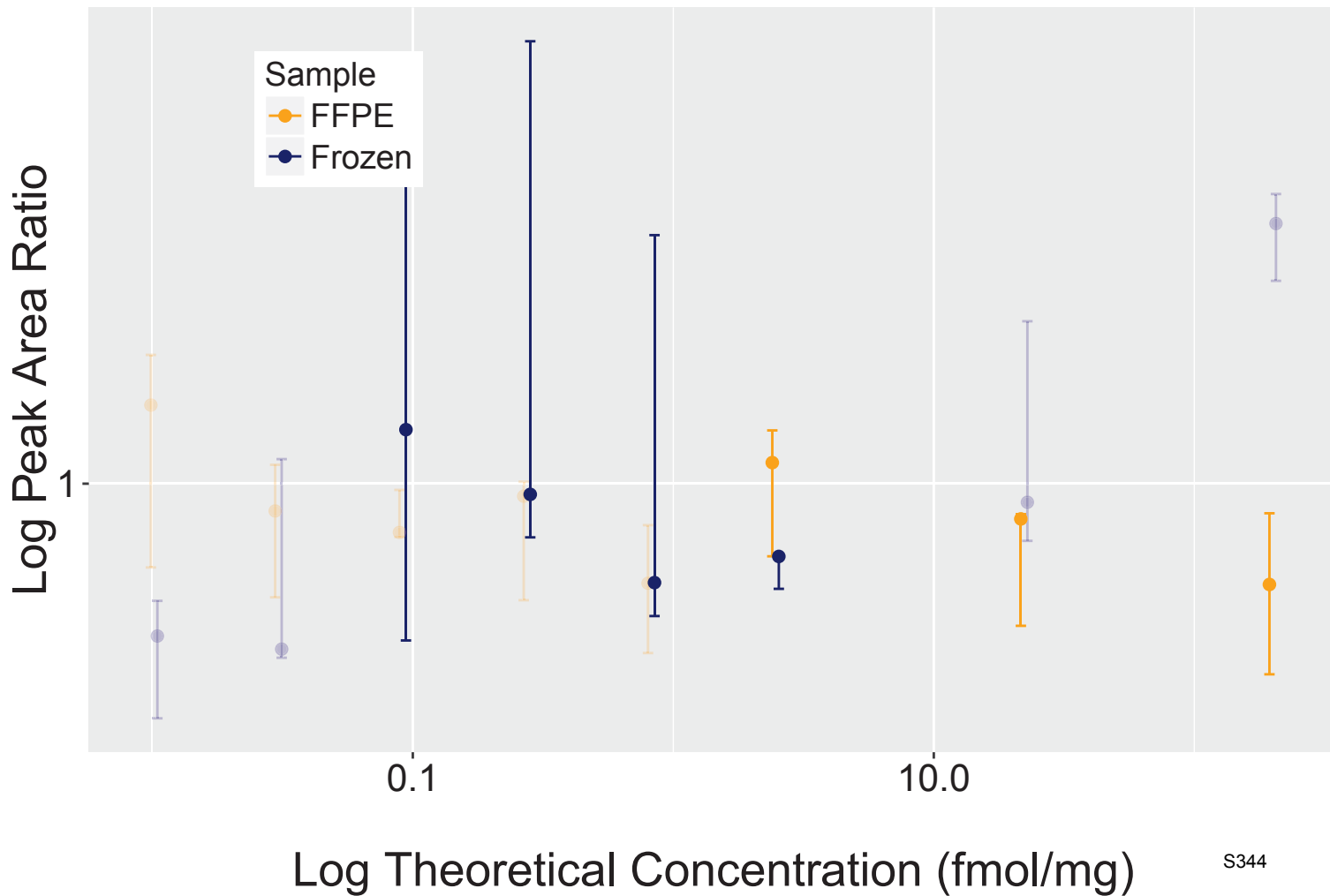
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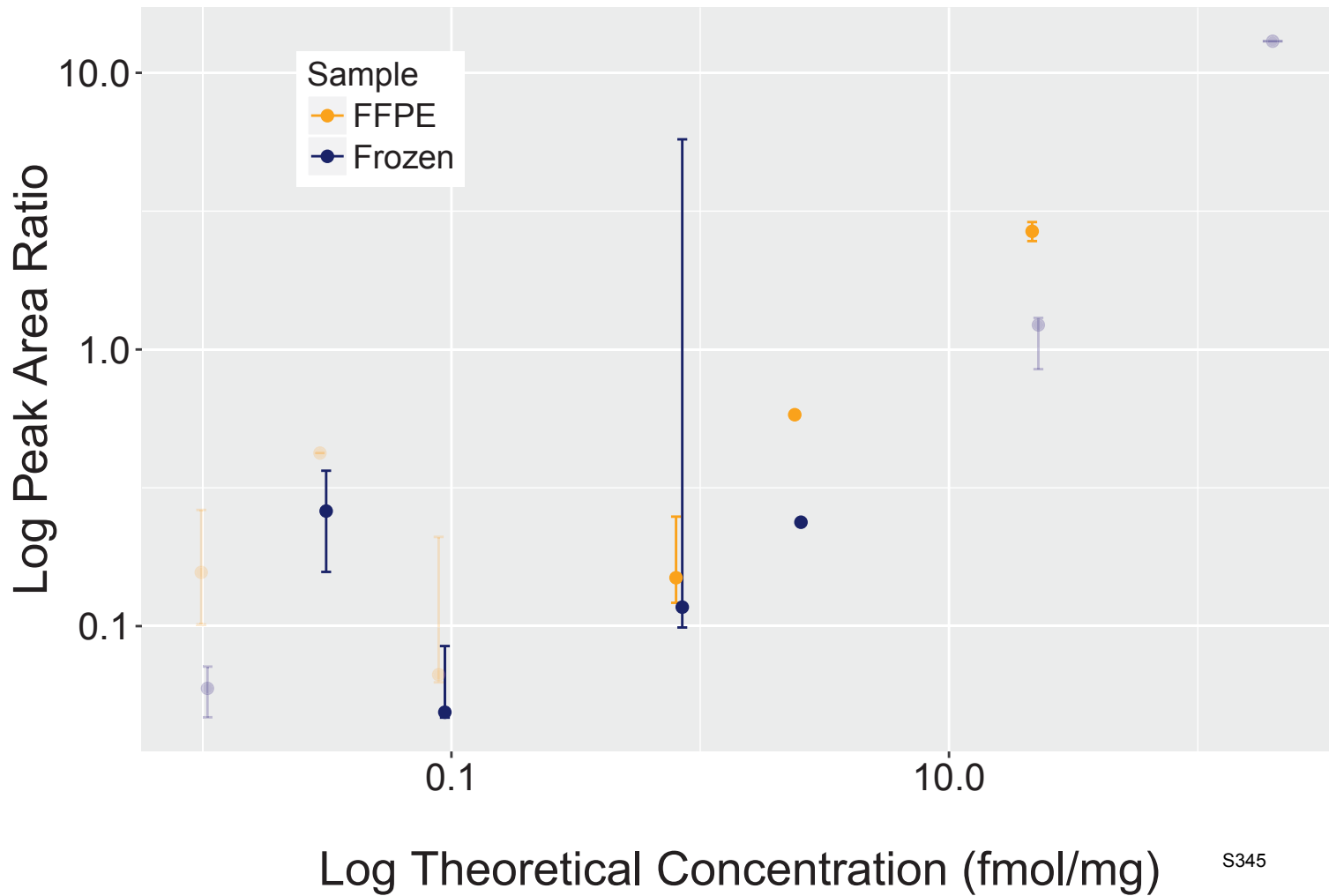
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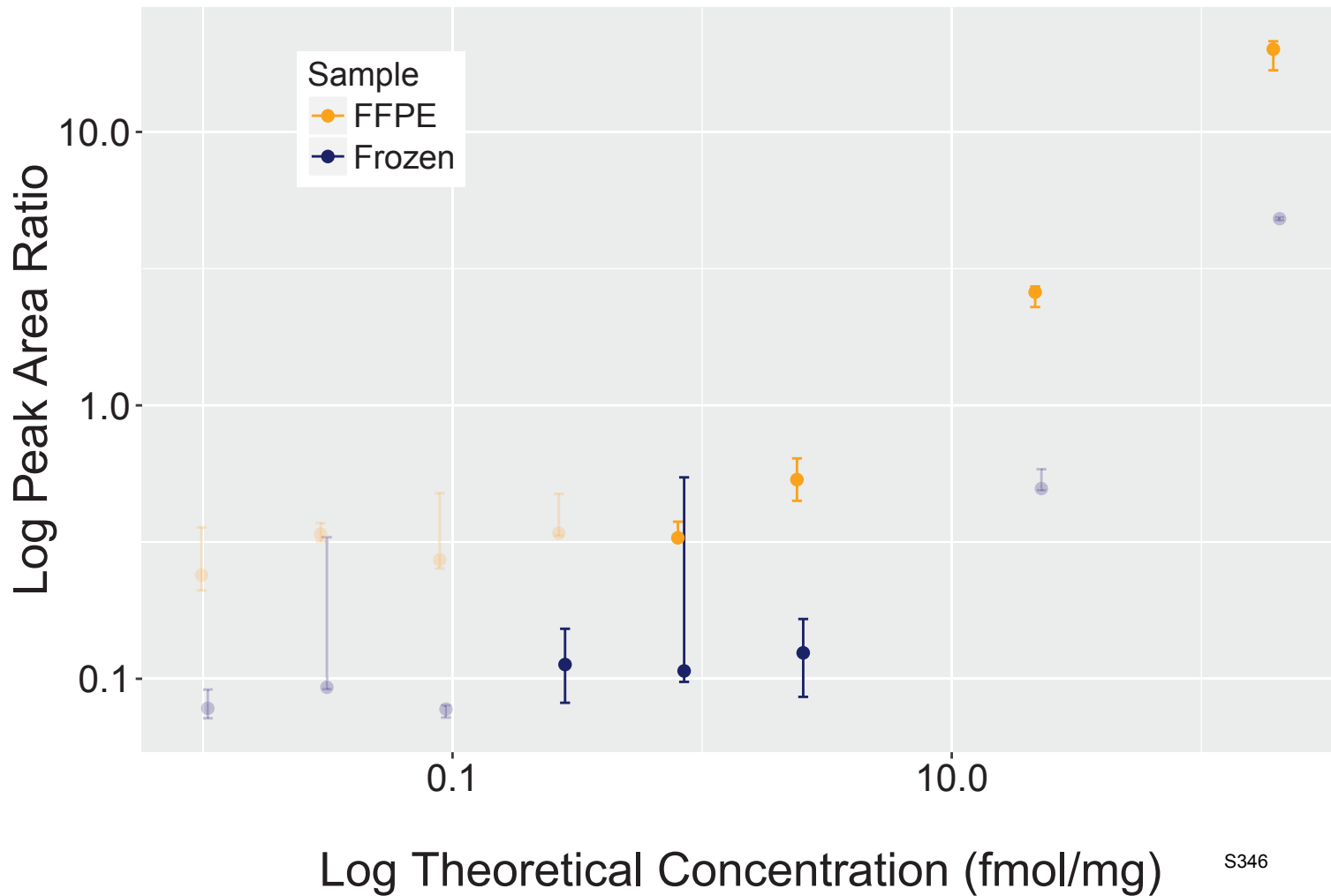
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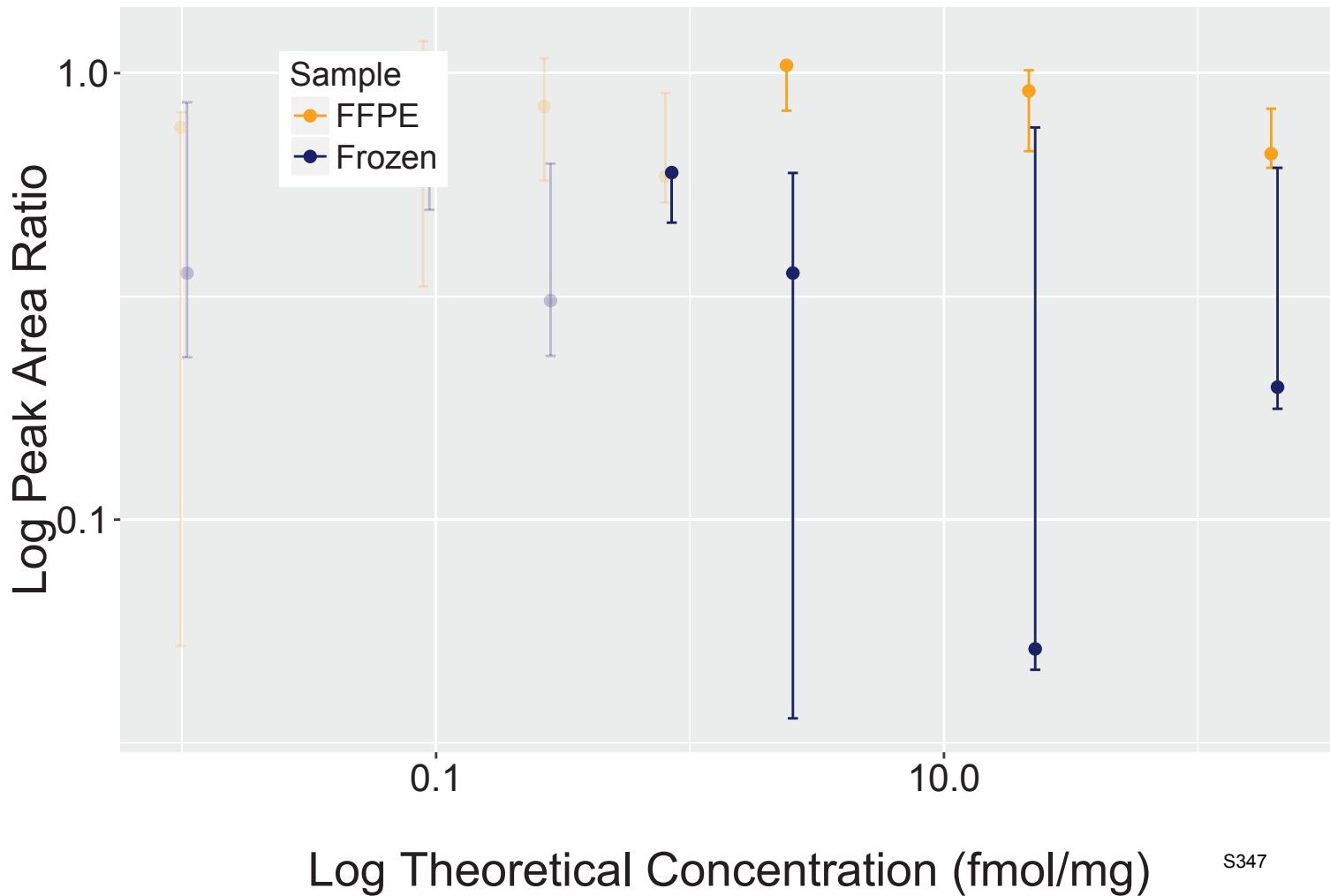
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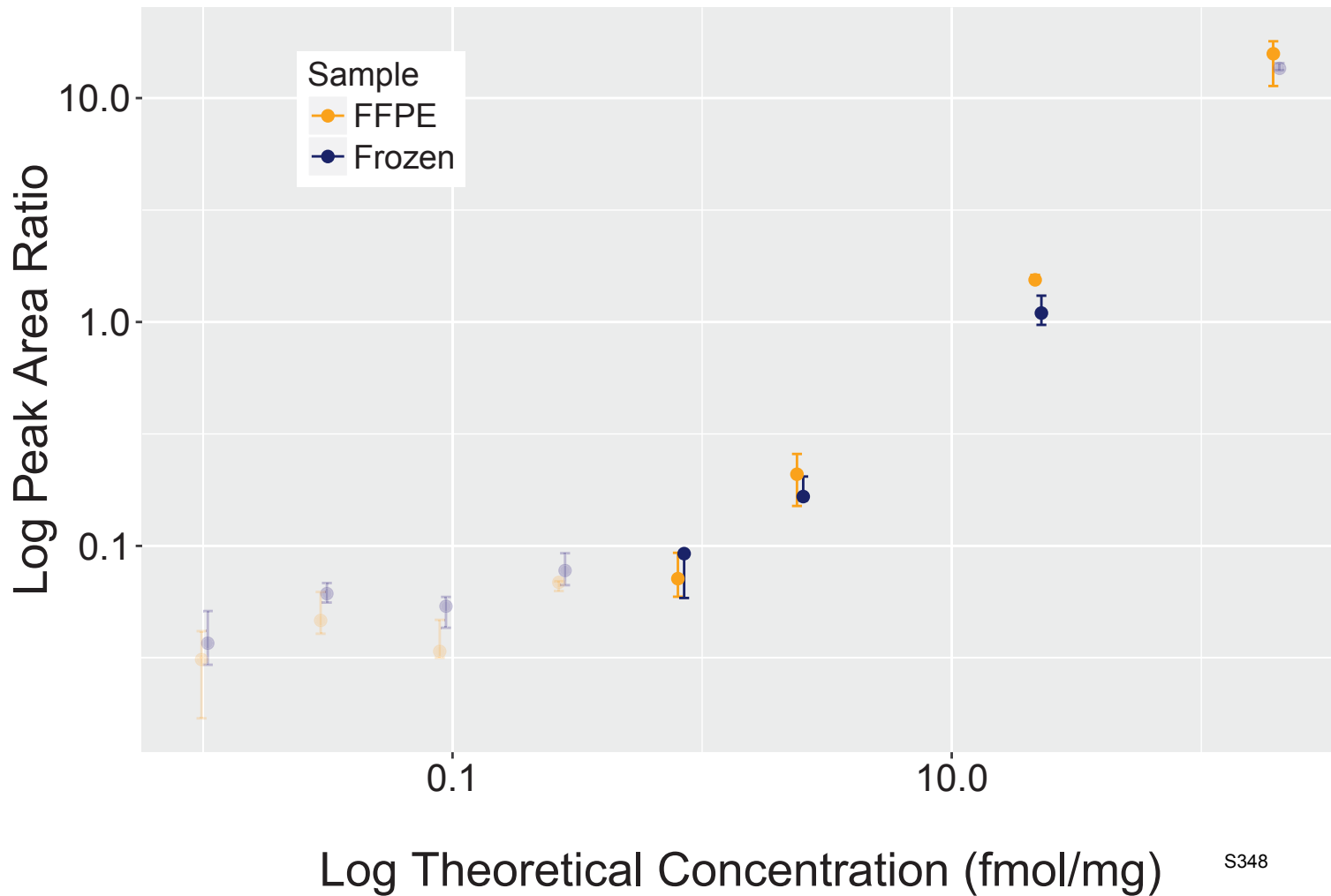
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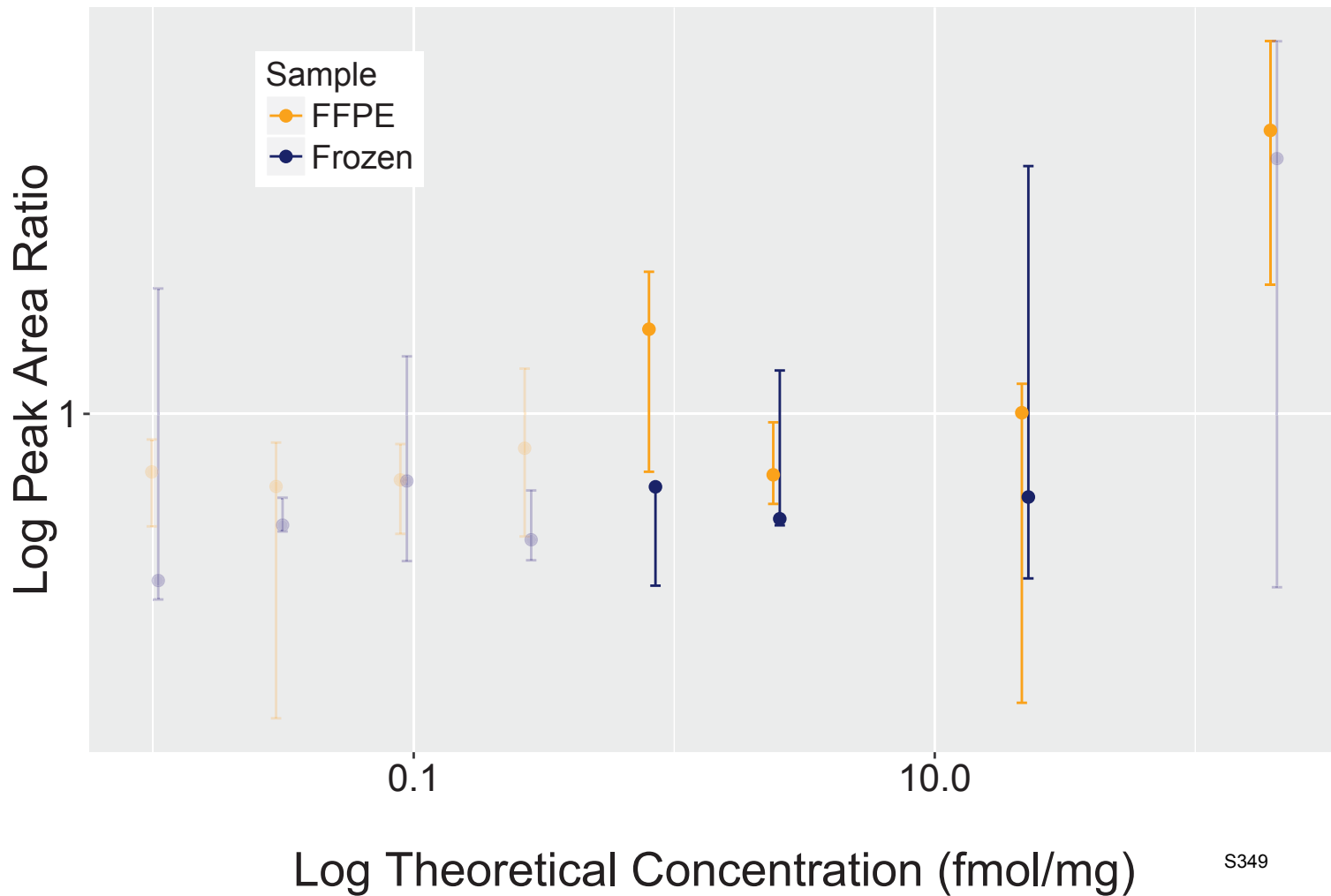
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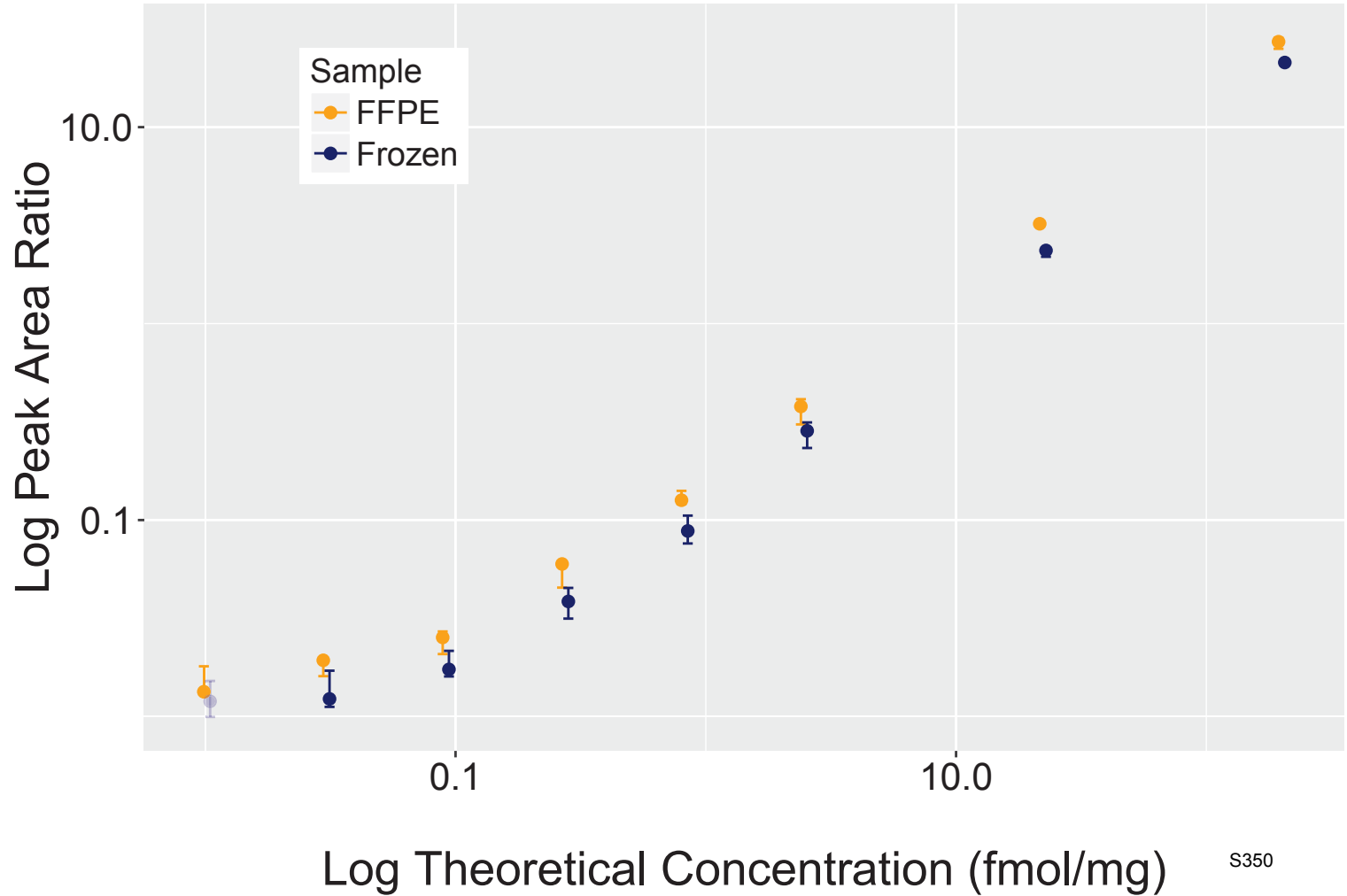
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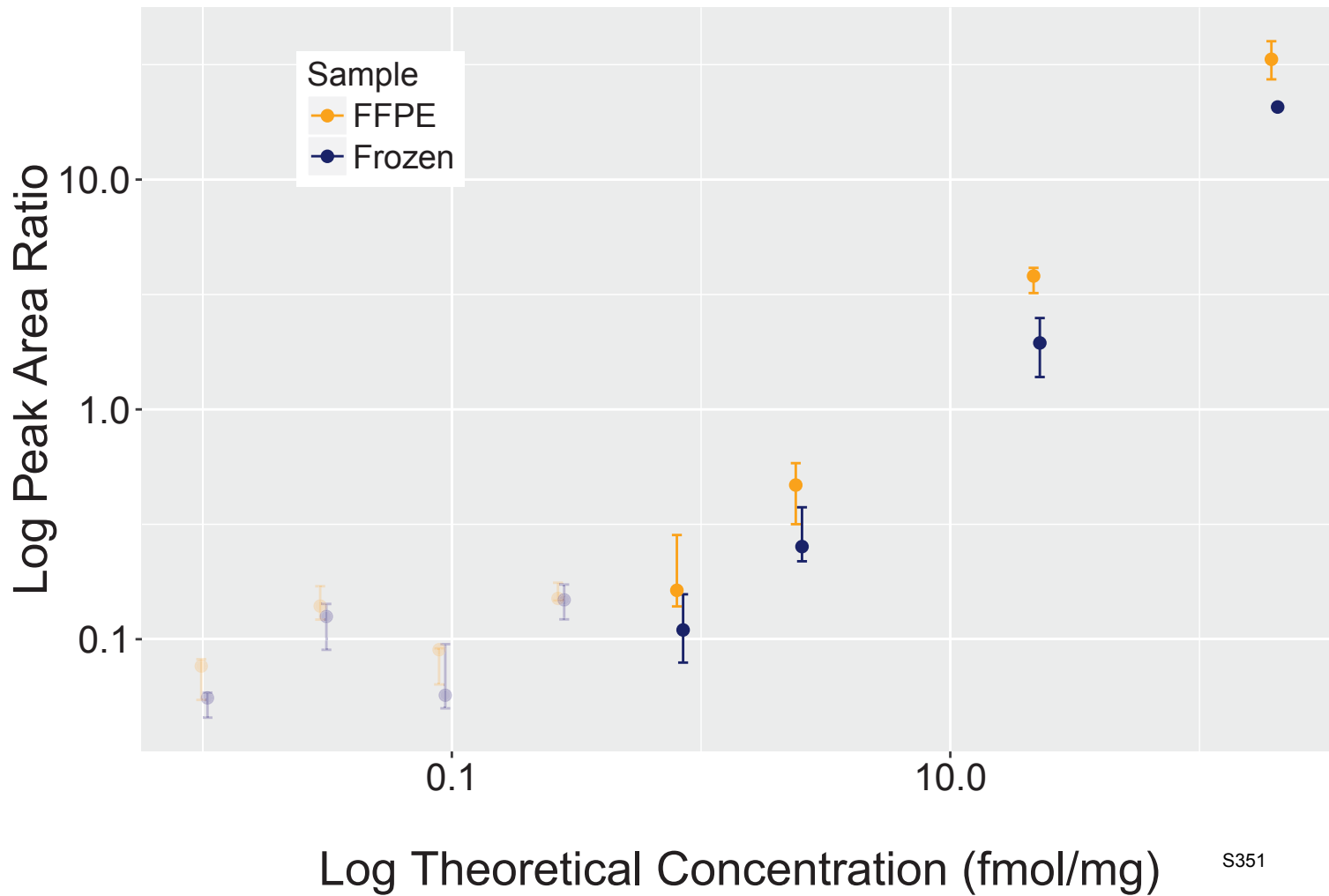
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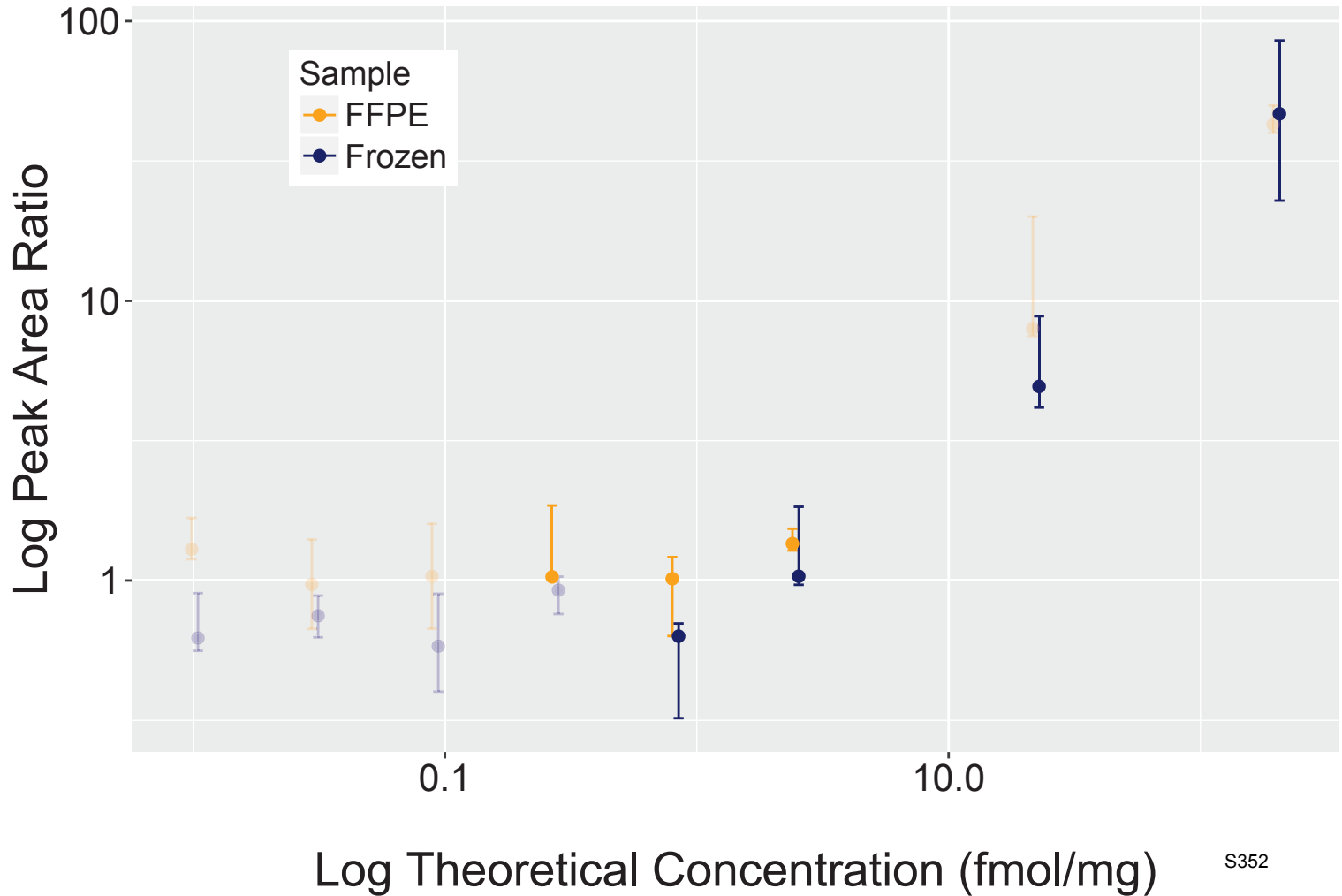
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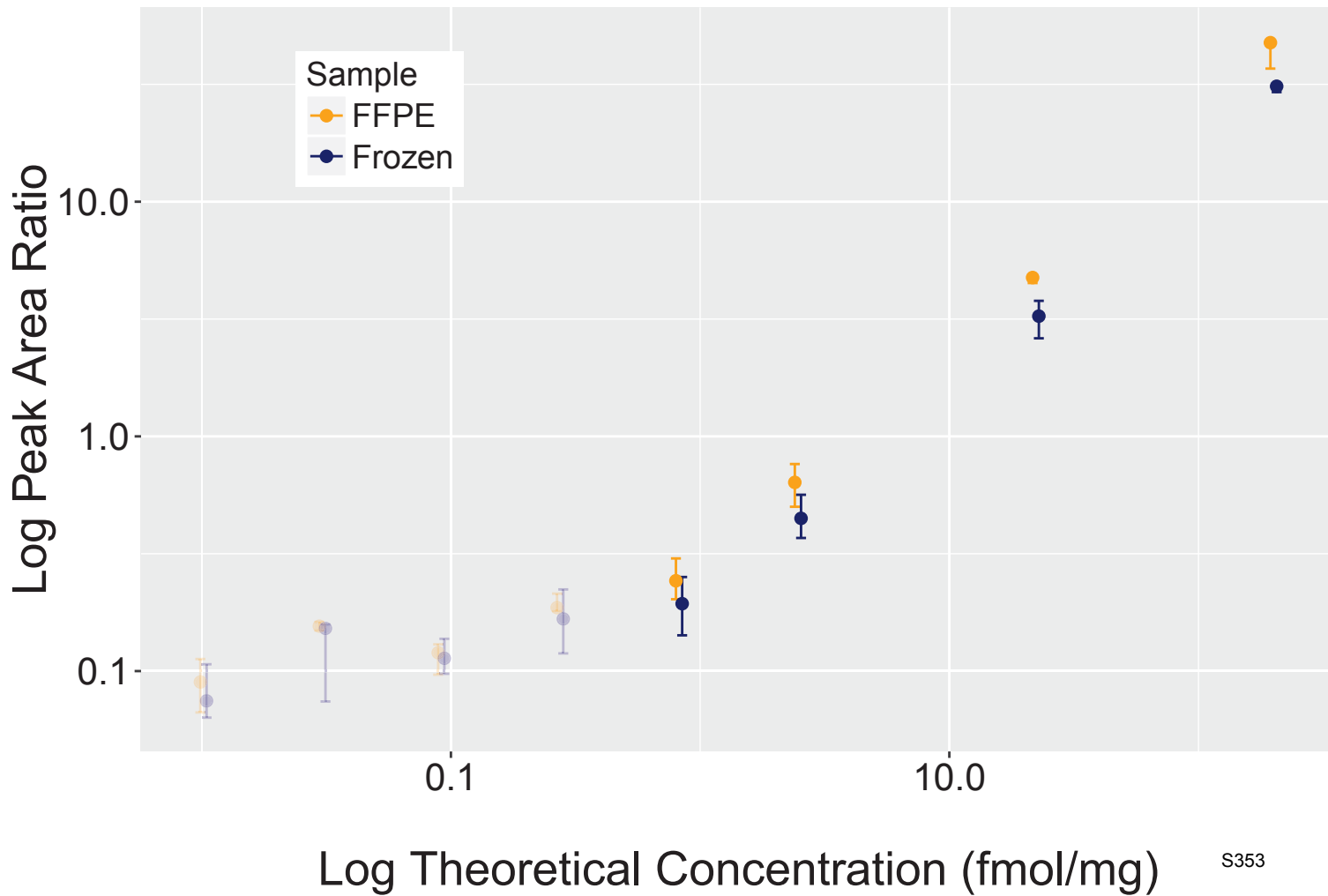
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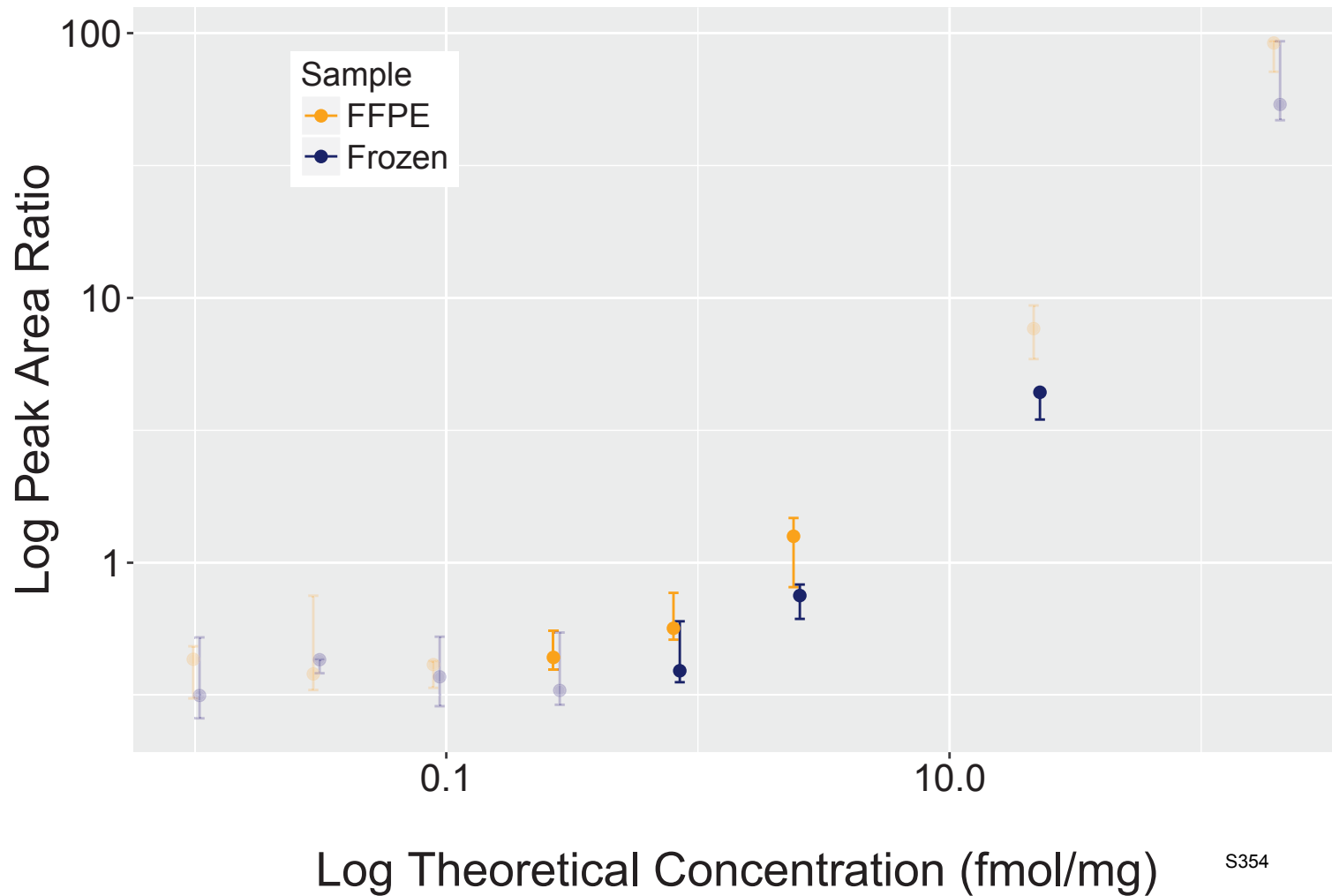
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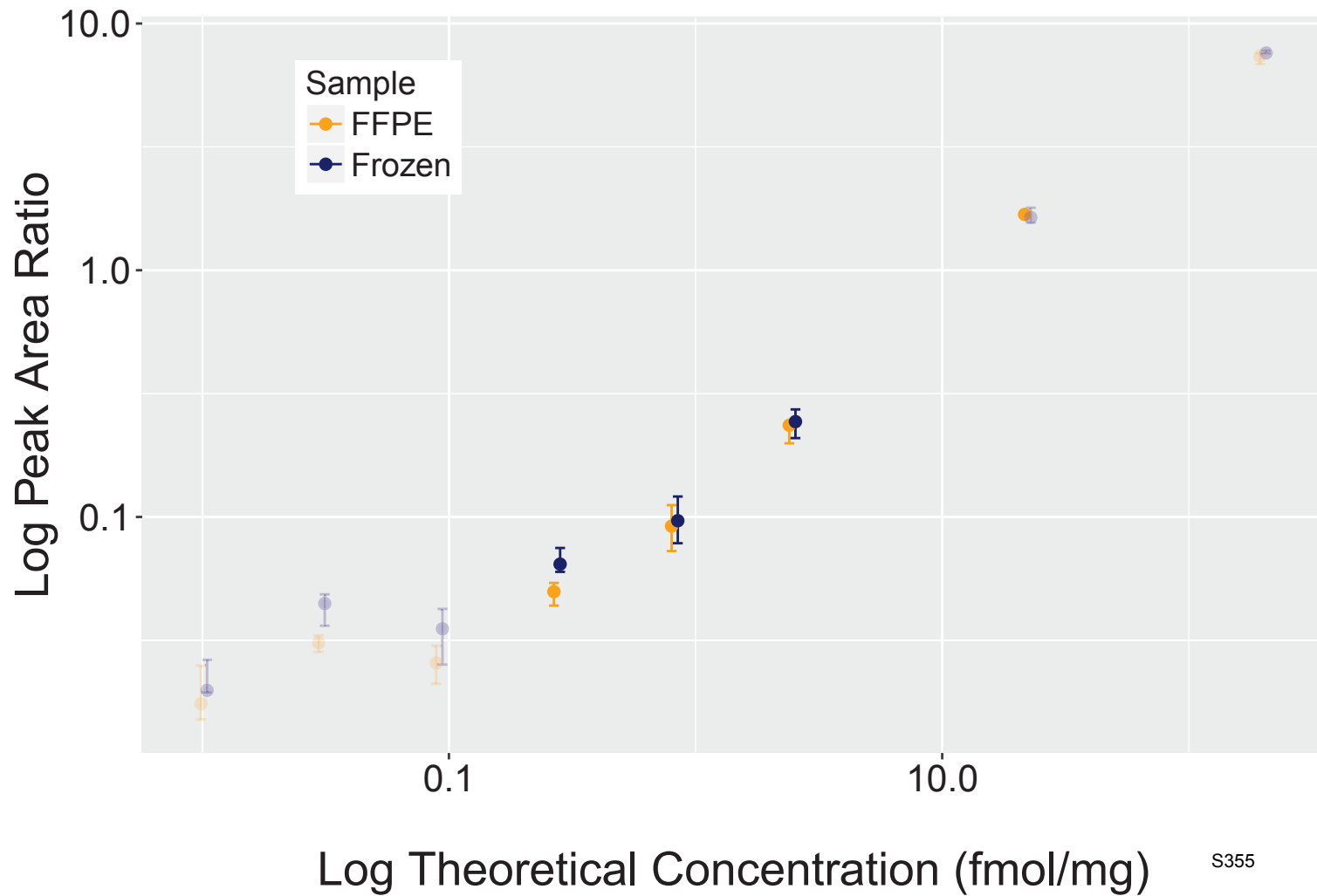
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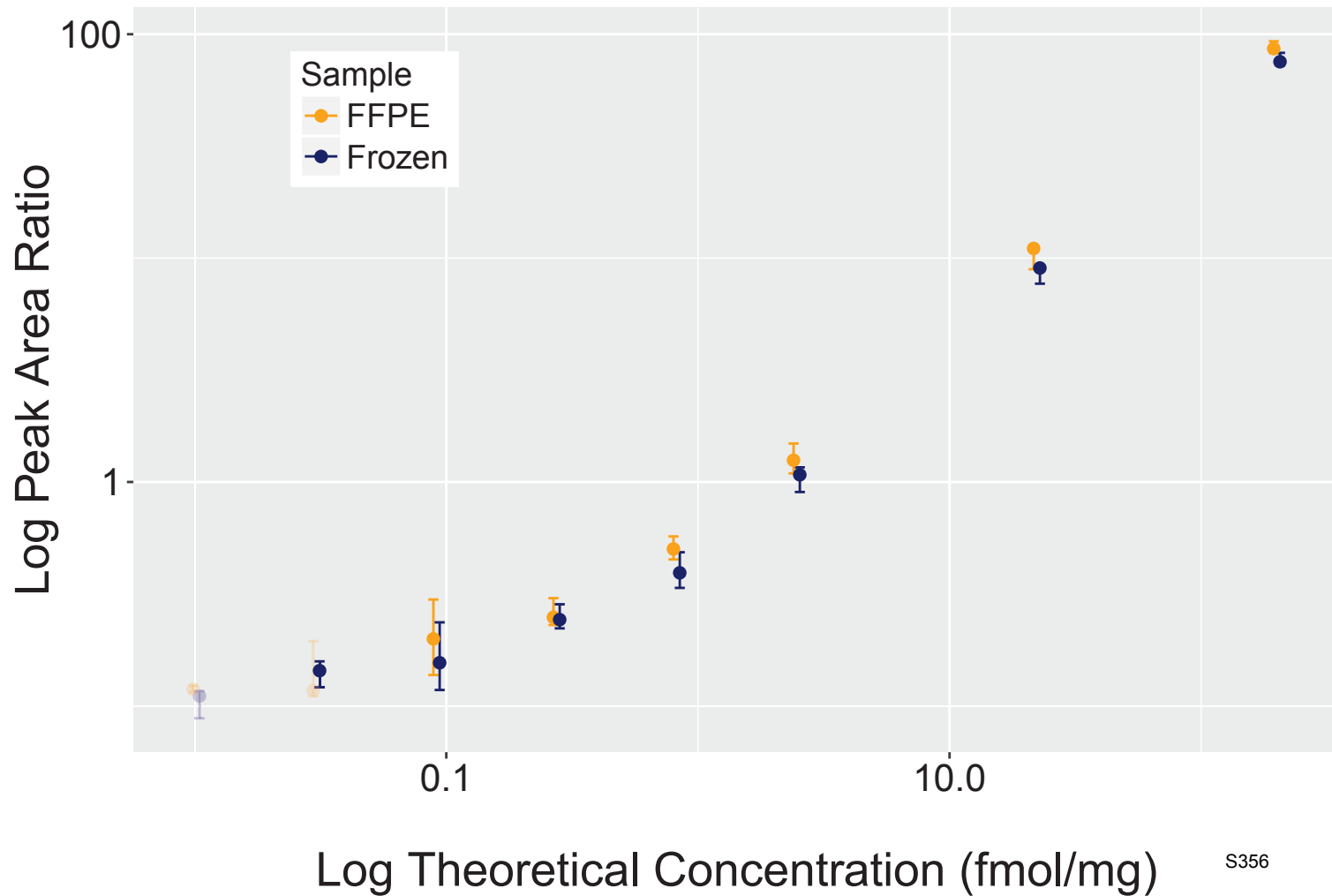
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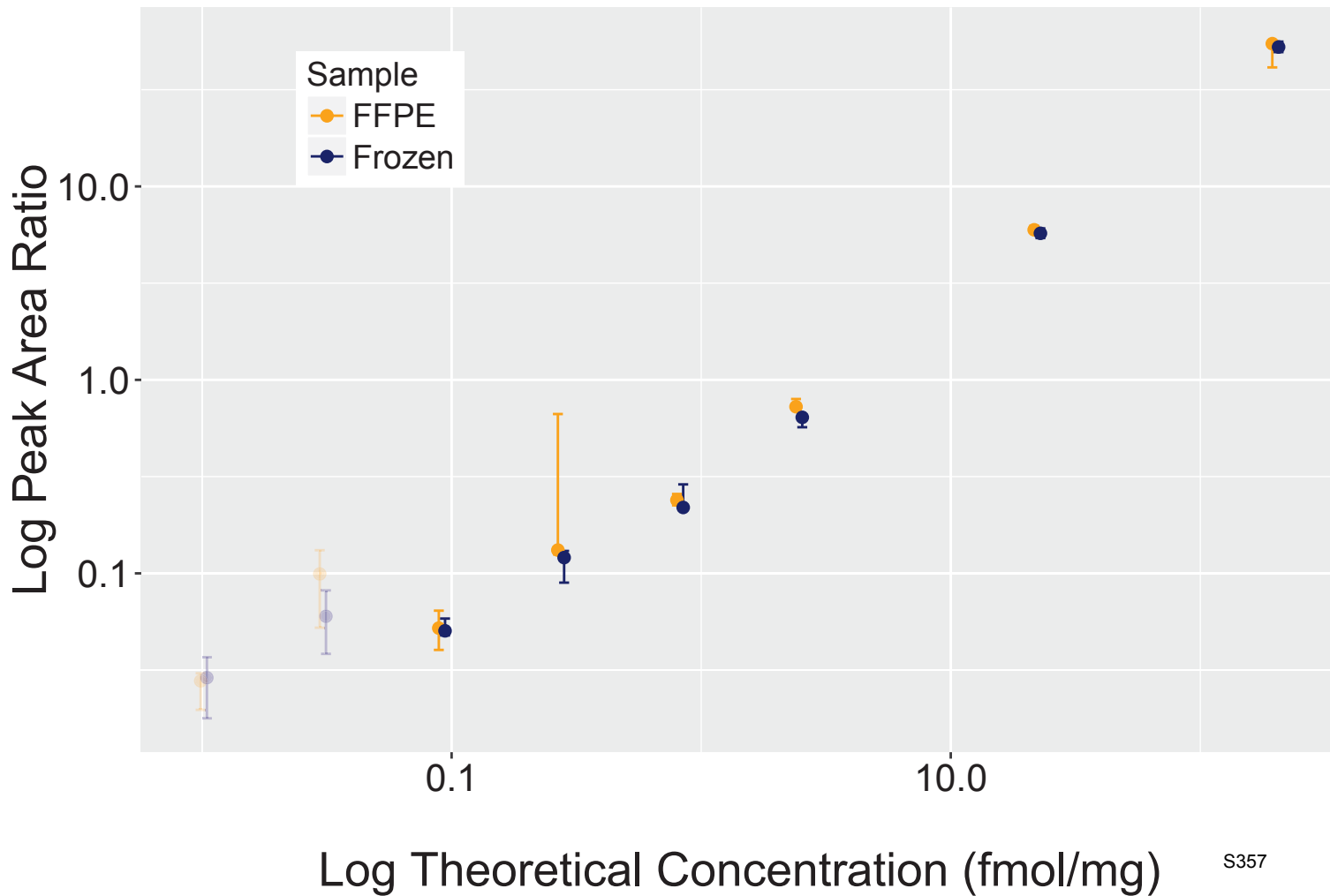
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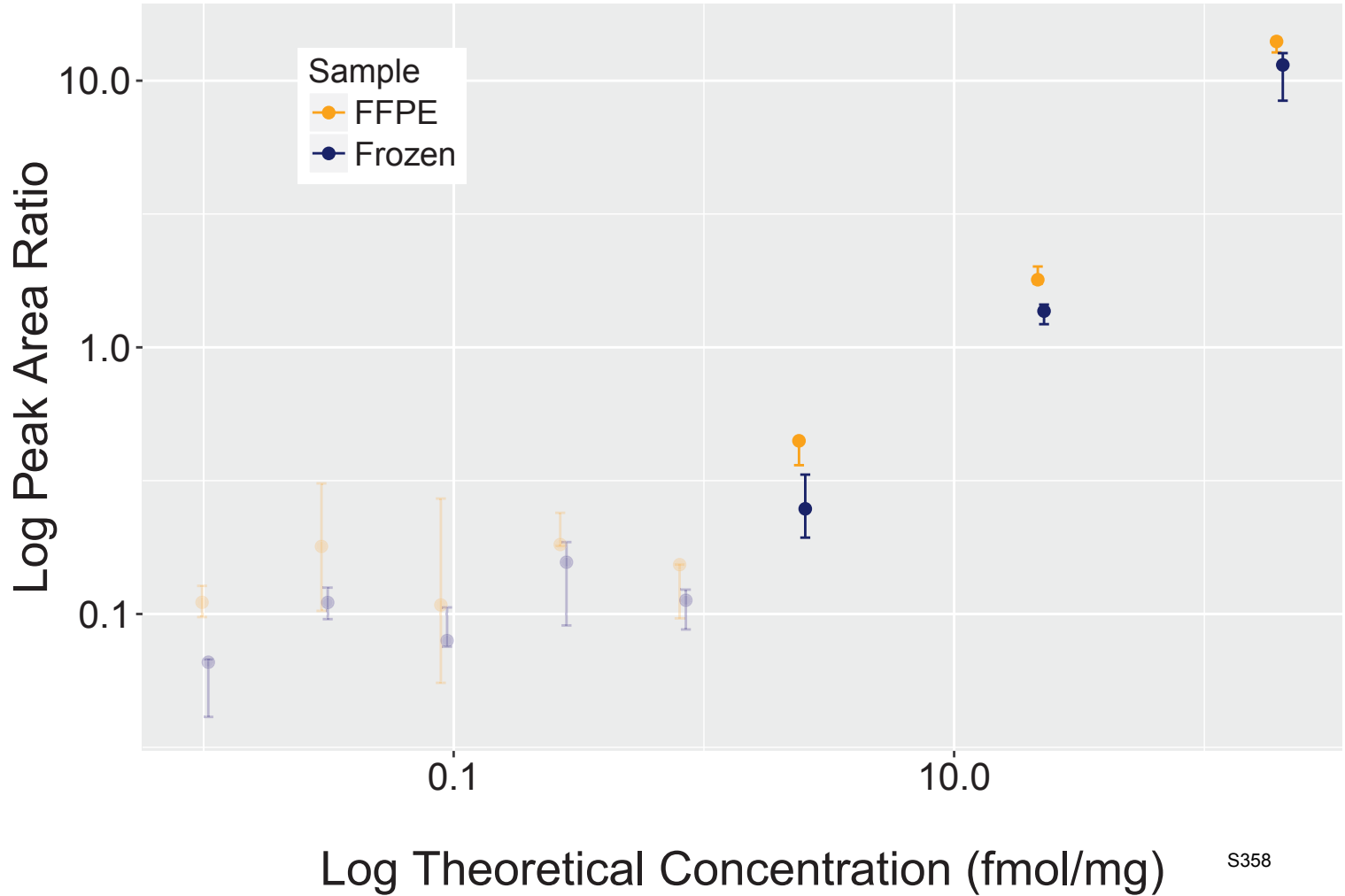
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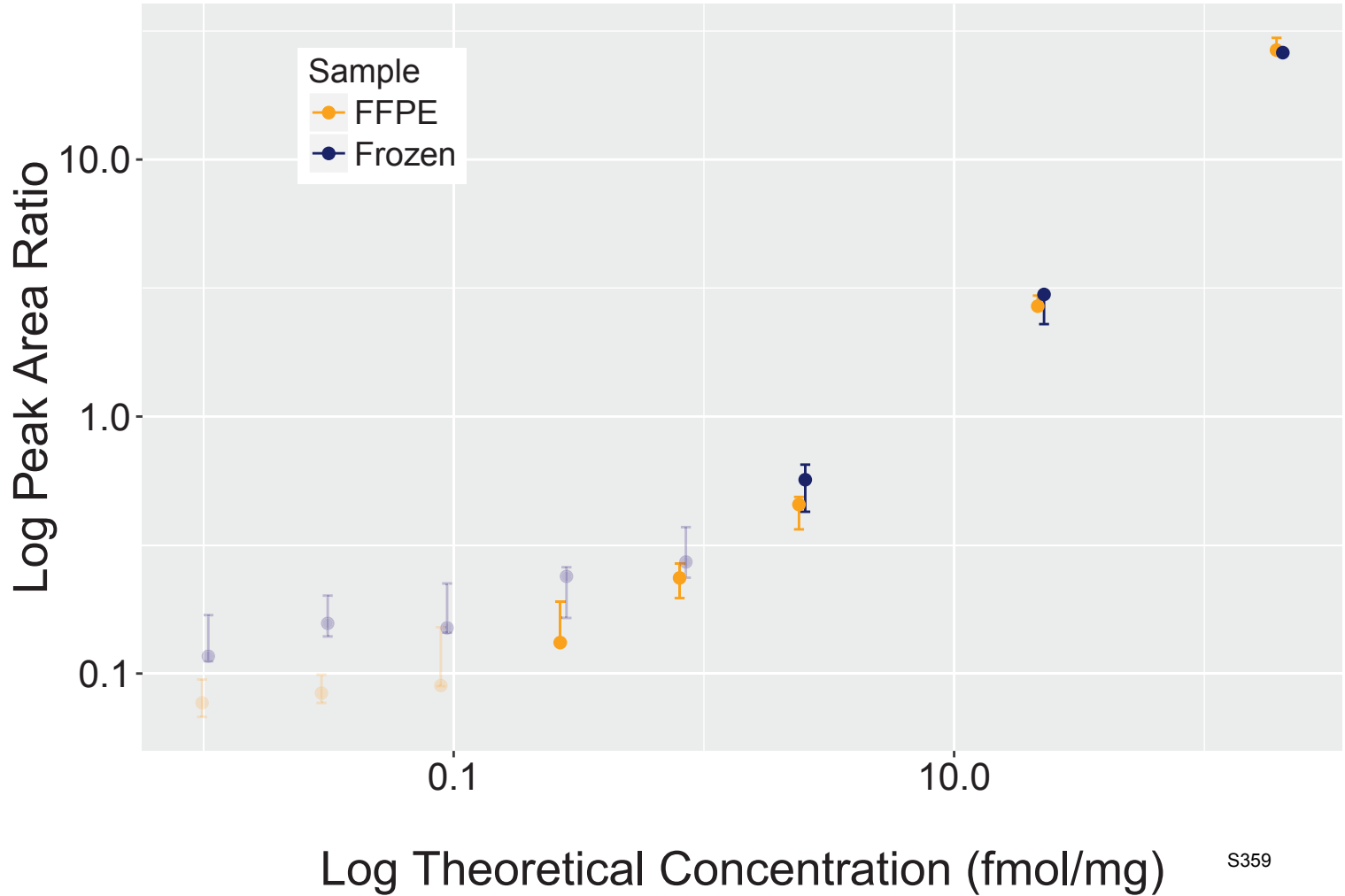
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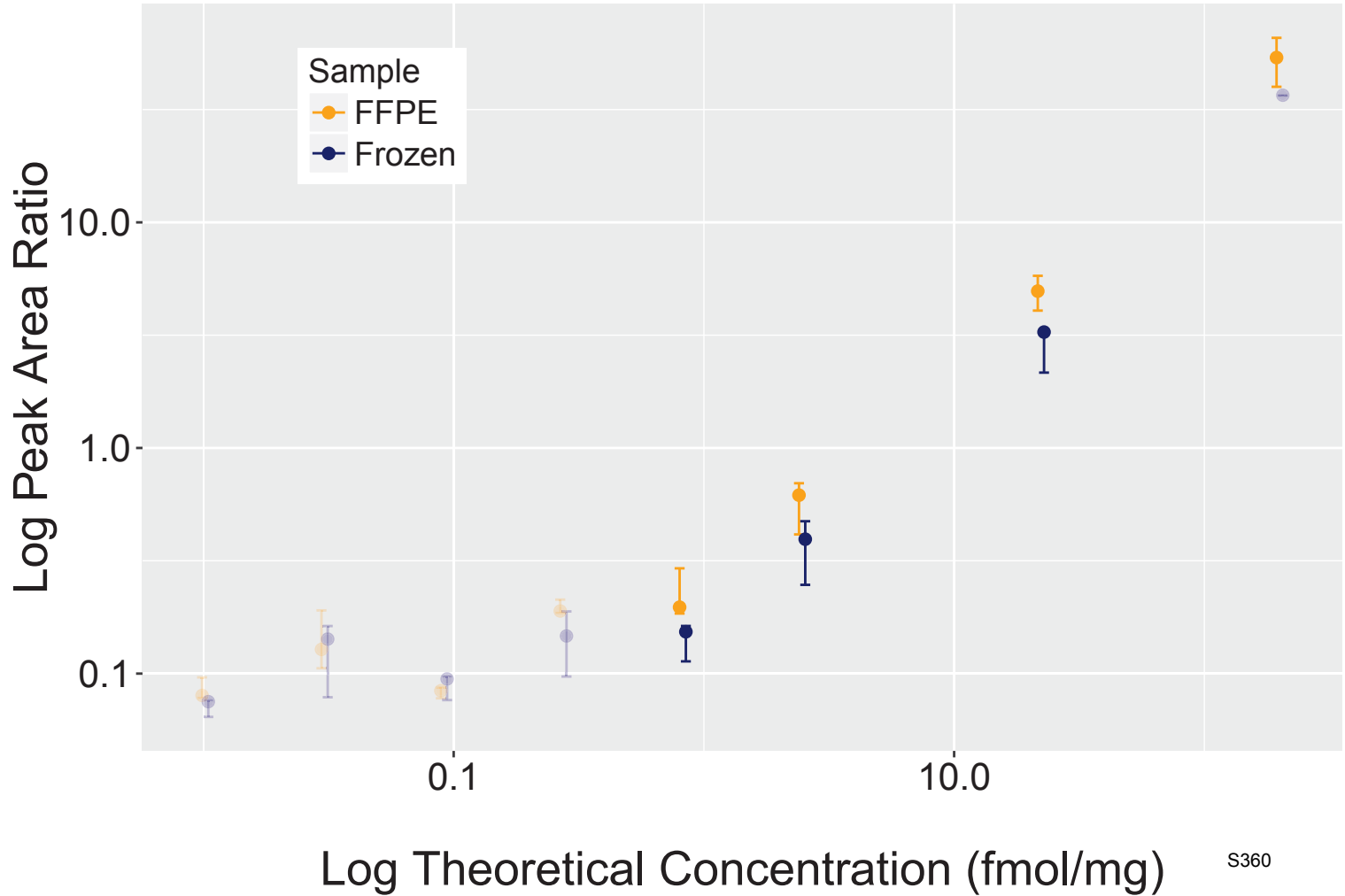
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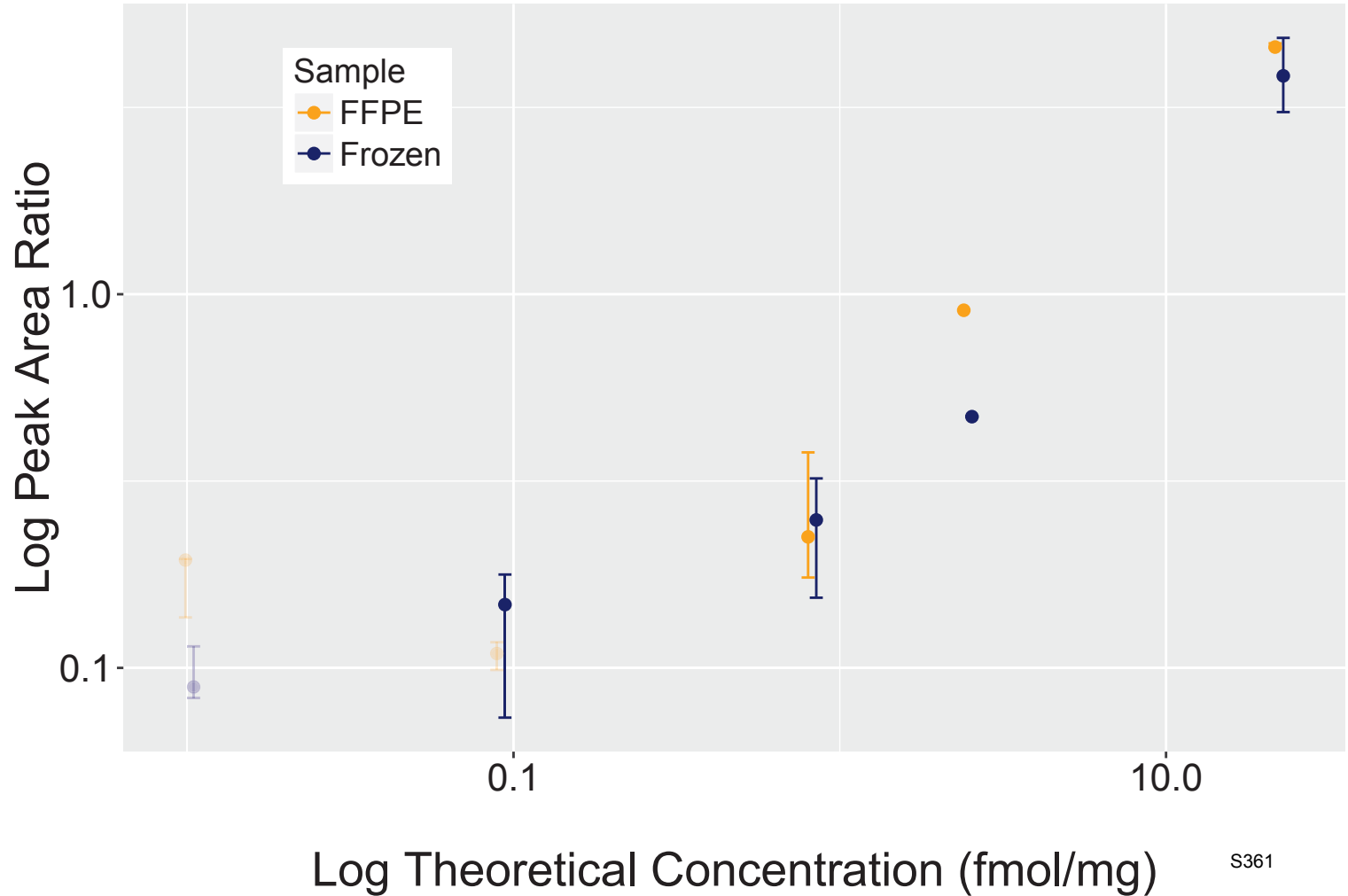
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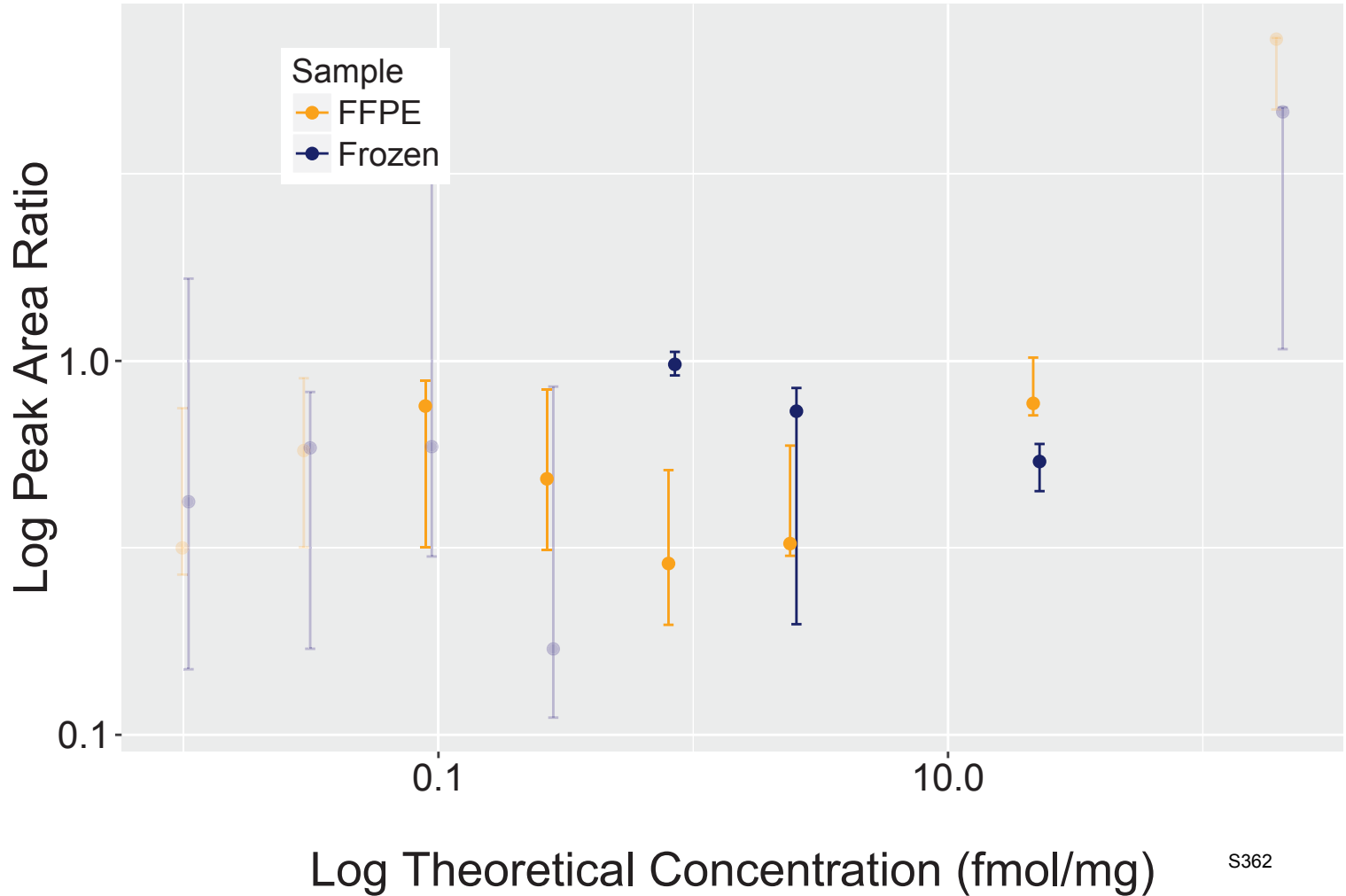
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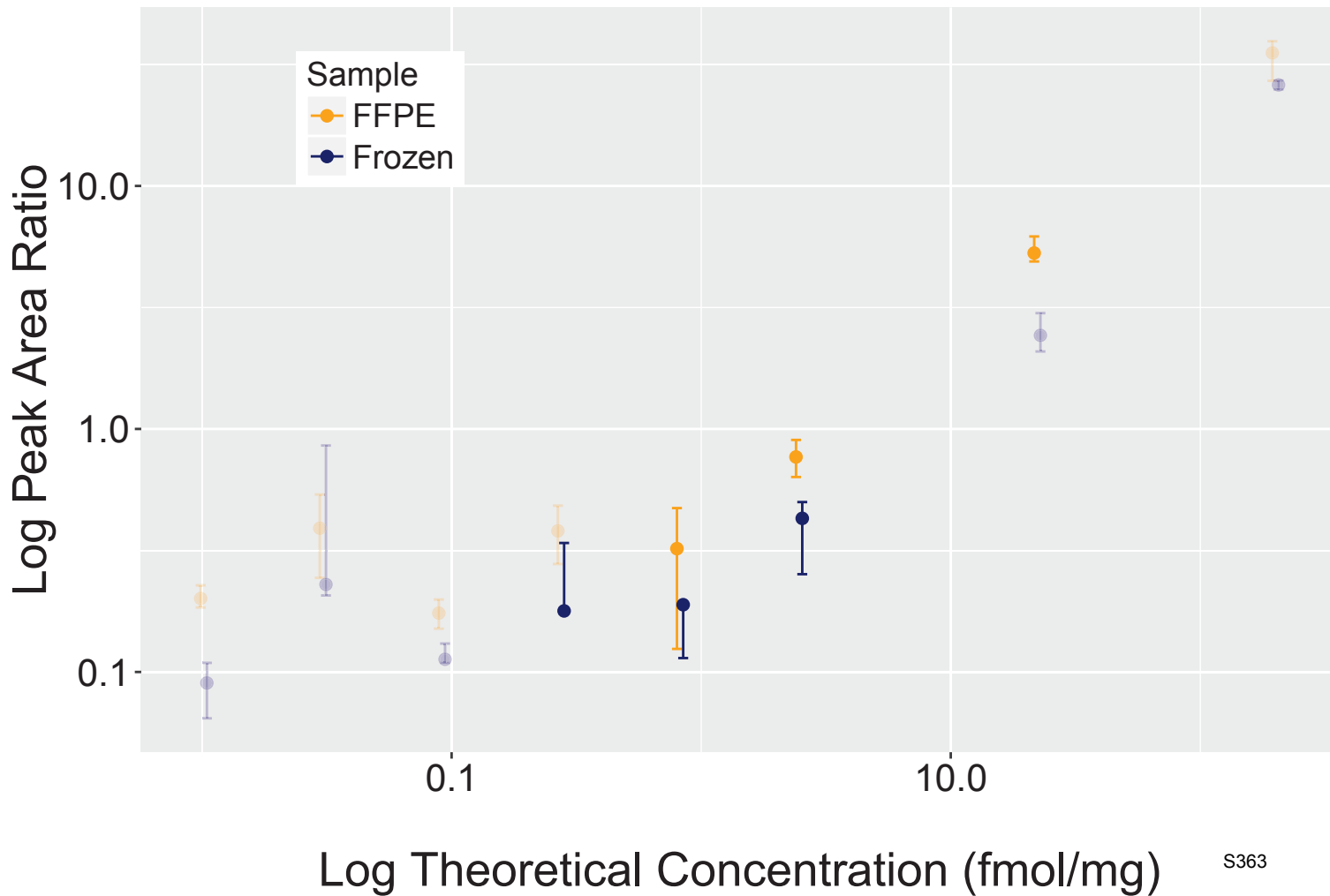
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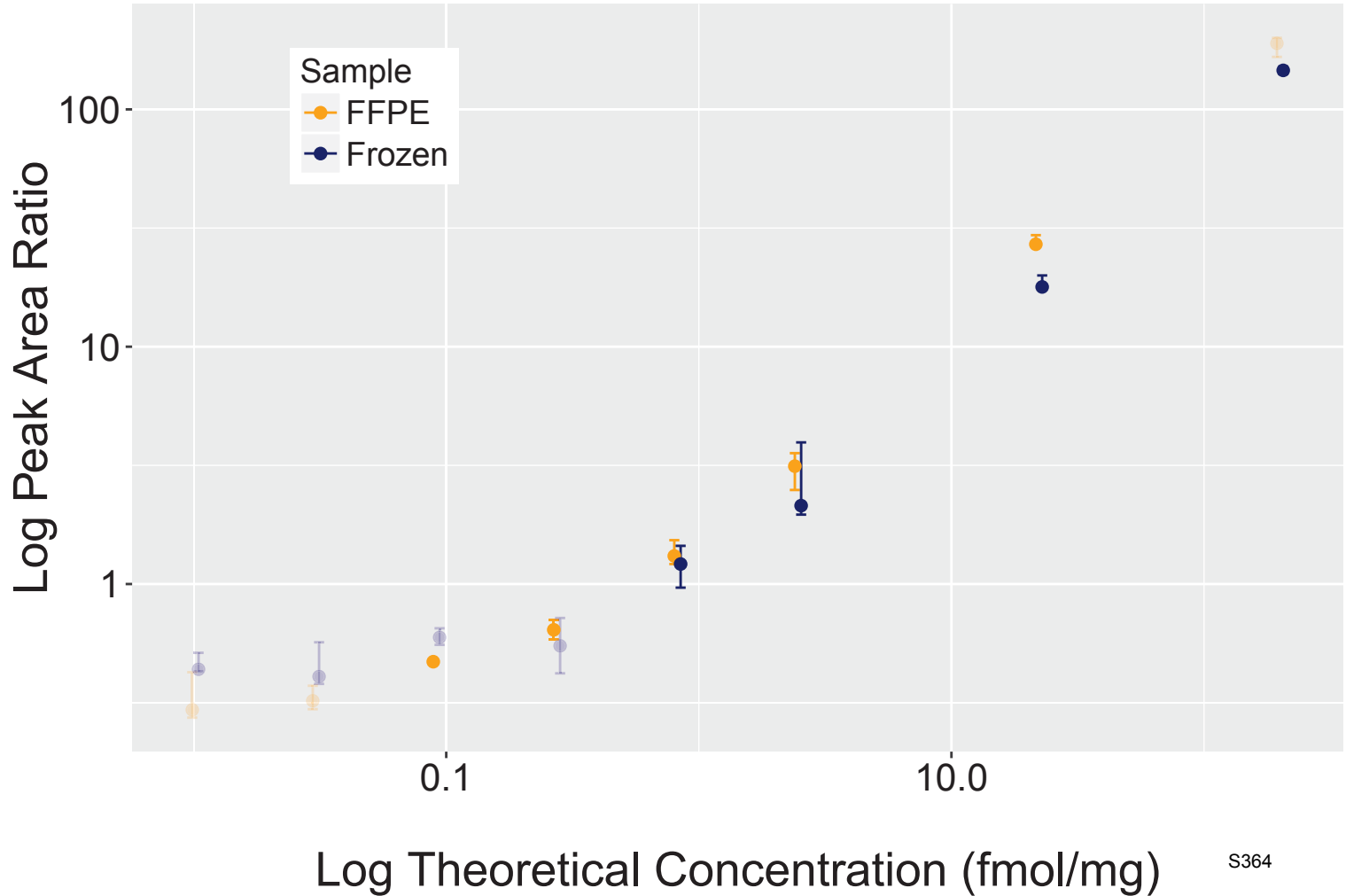
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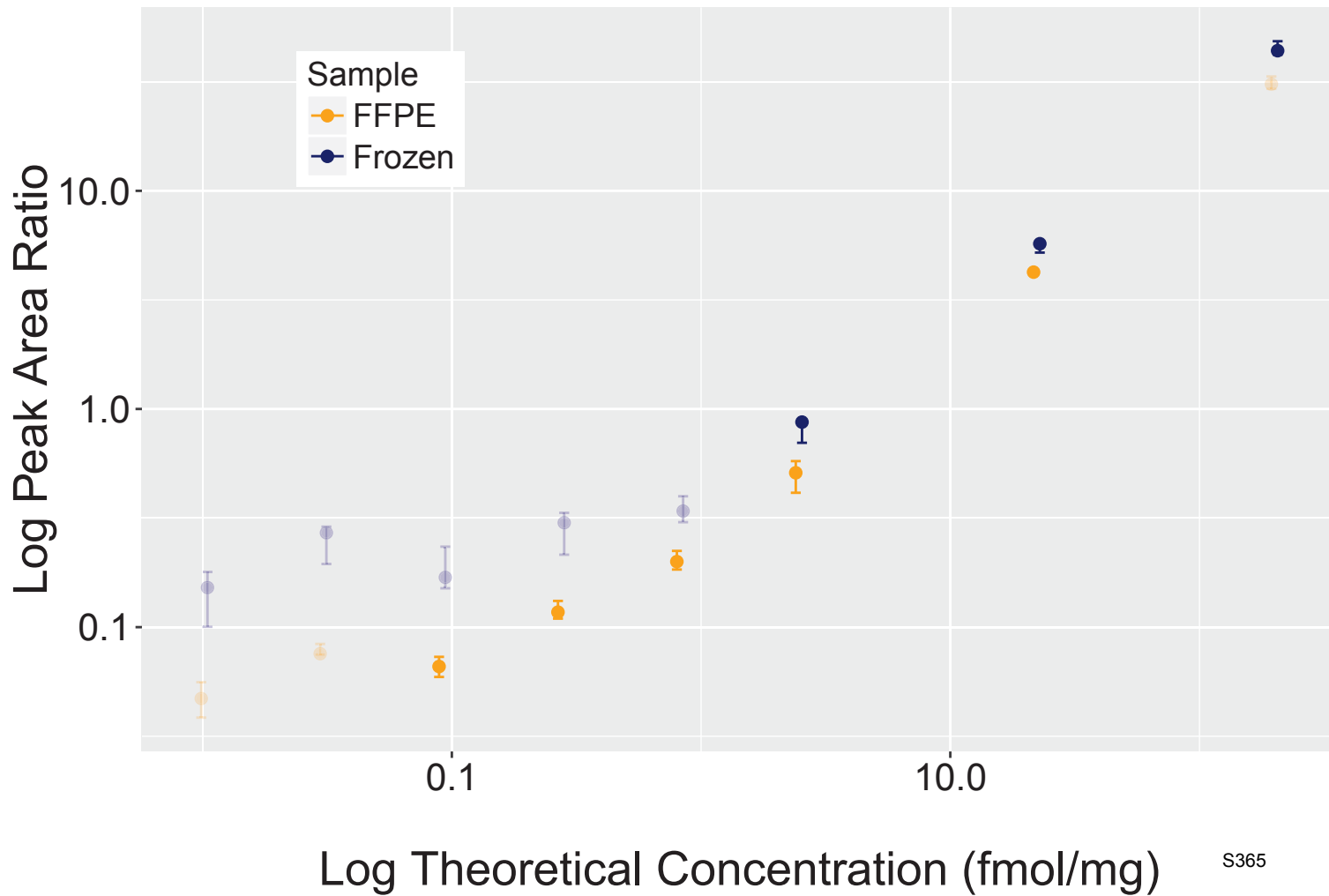
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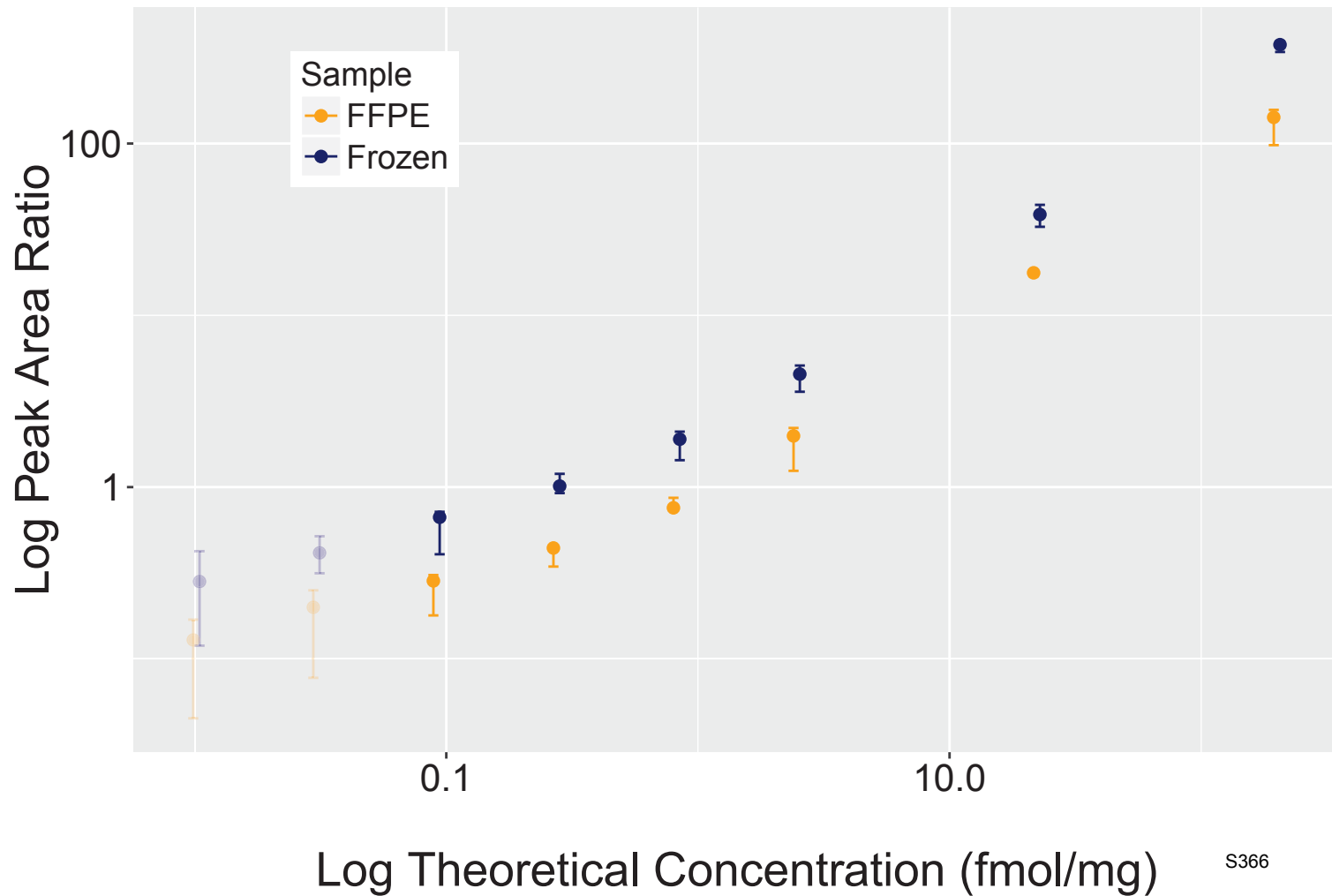
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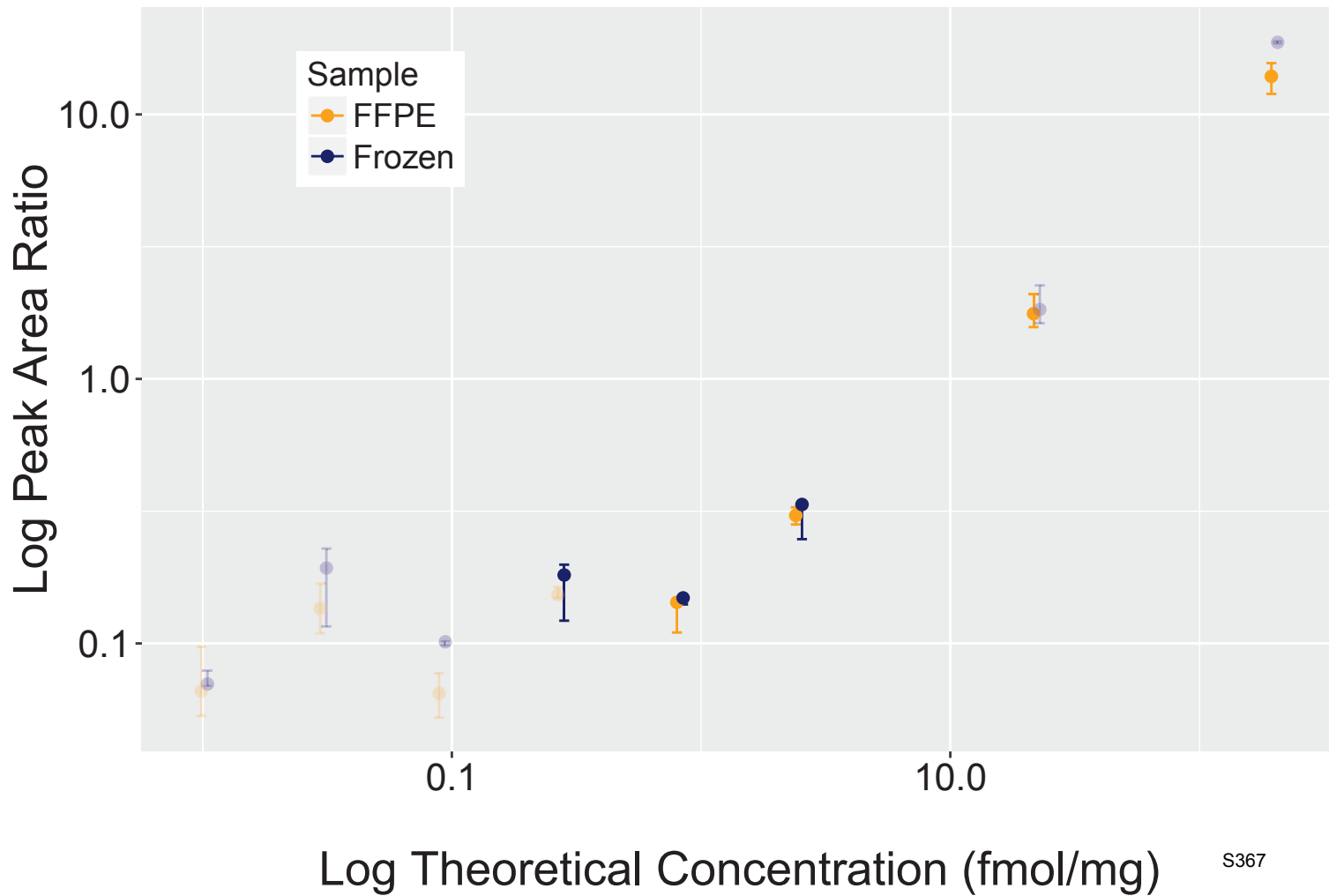
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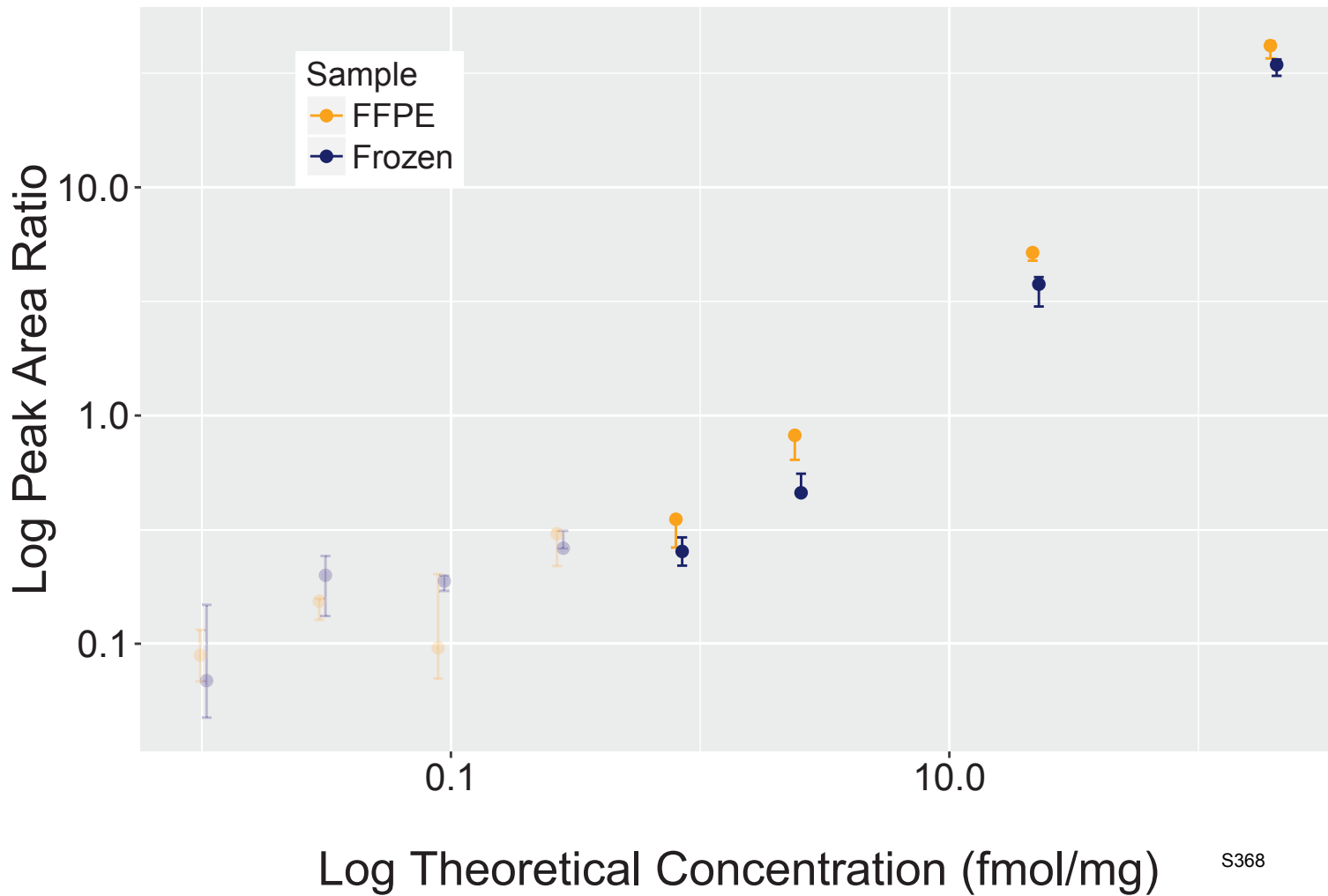
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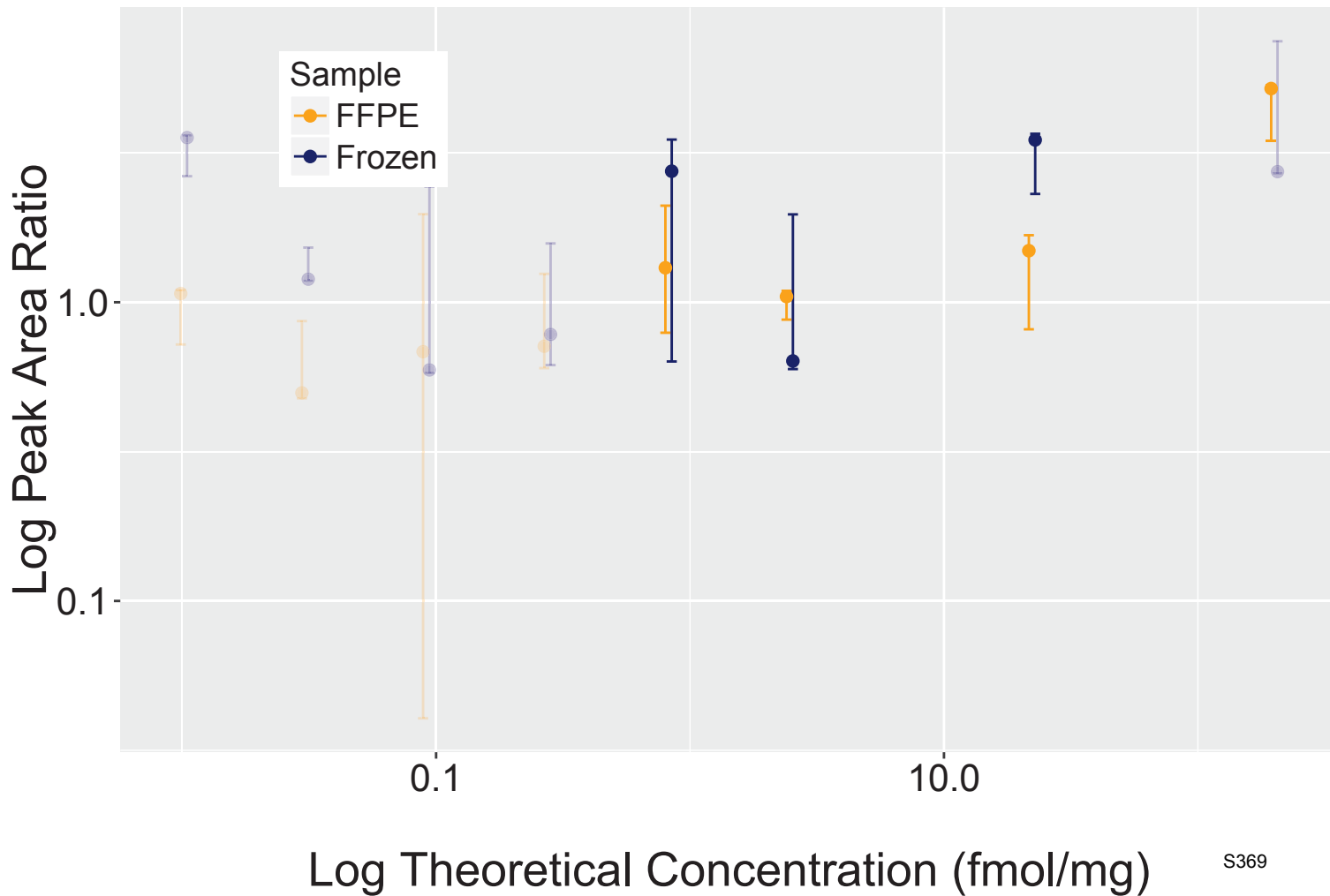
Analyte: CKAP4.LEGLGSSEADQDGLASTVR



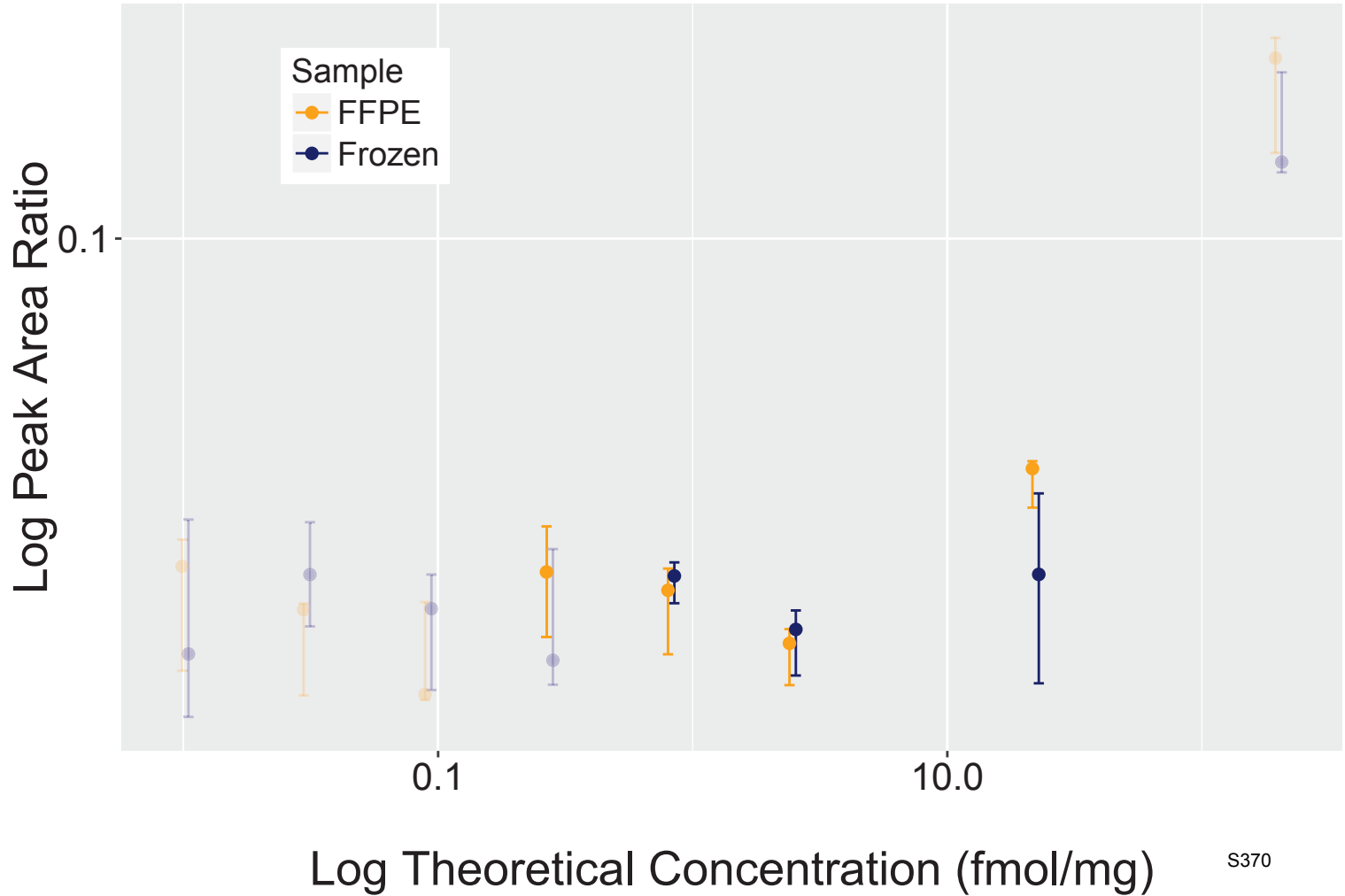
Analyte: CAPN1.LEIC[+57]NLTPDALK



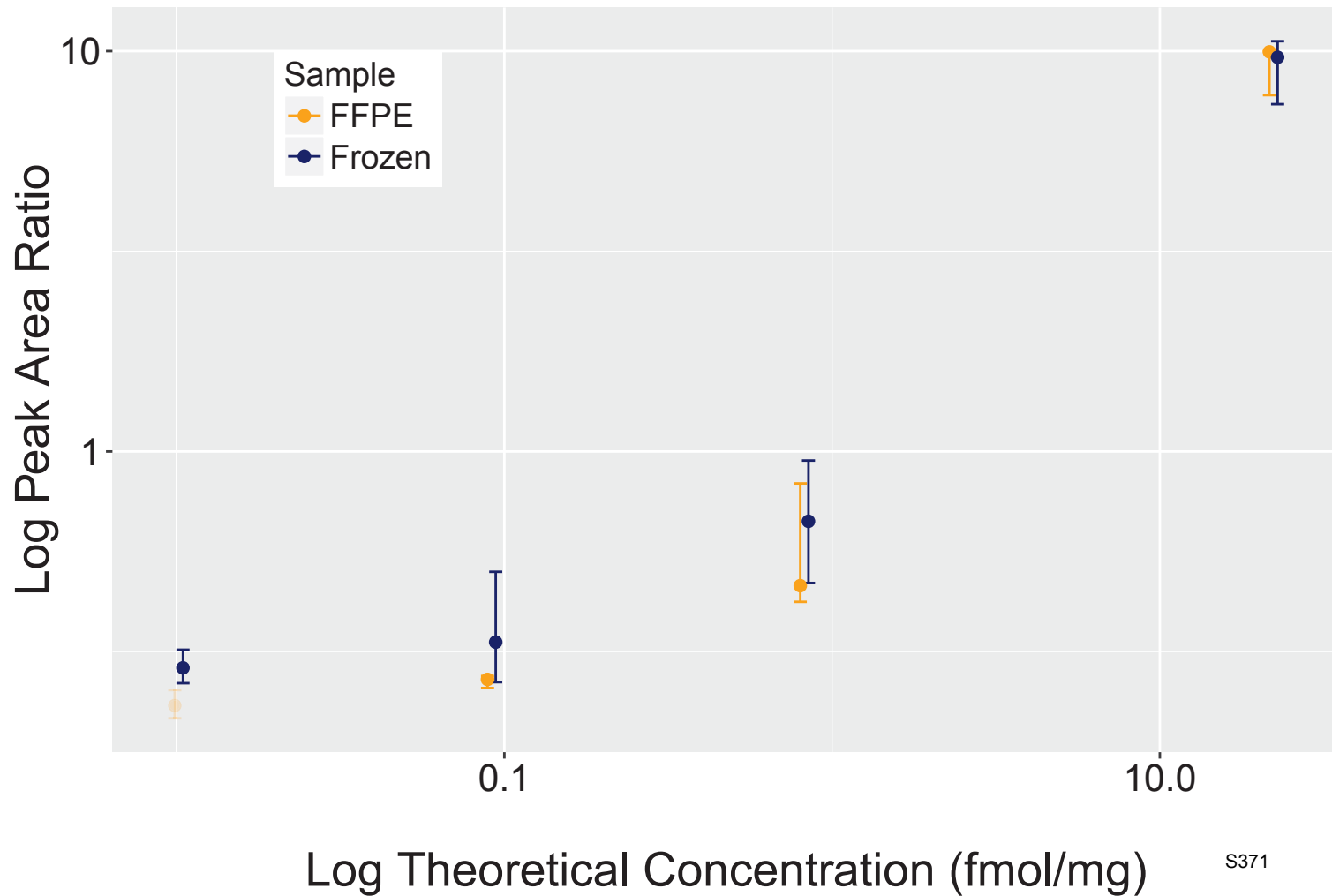
Analyte: CALD1.LEQY TSAIEGTK



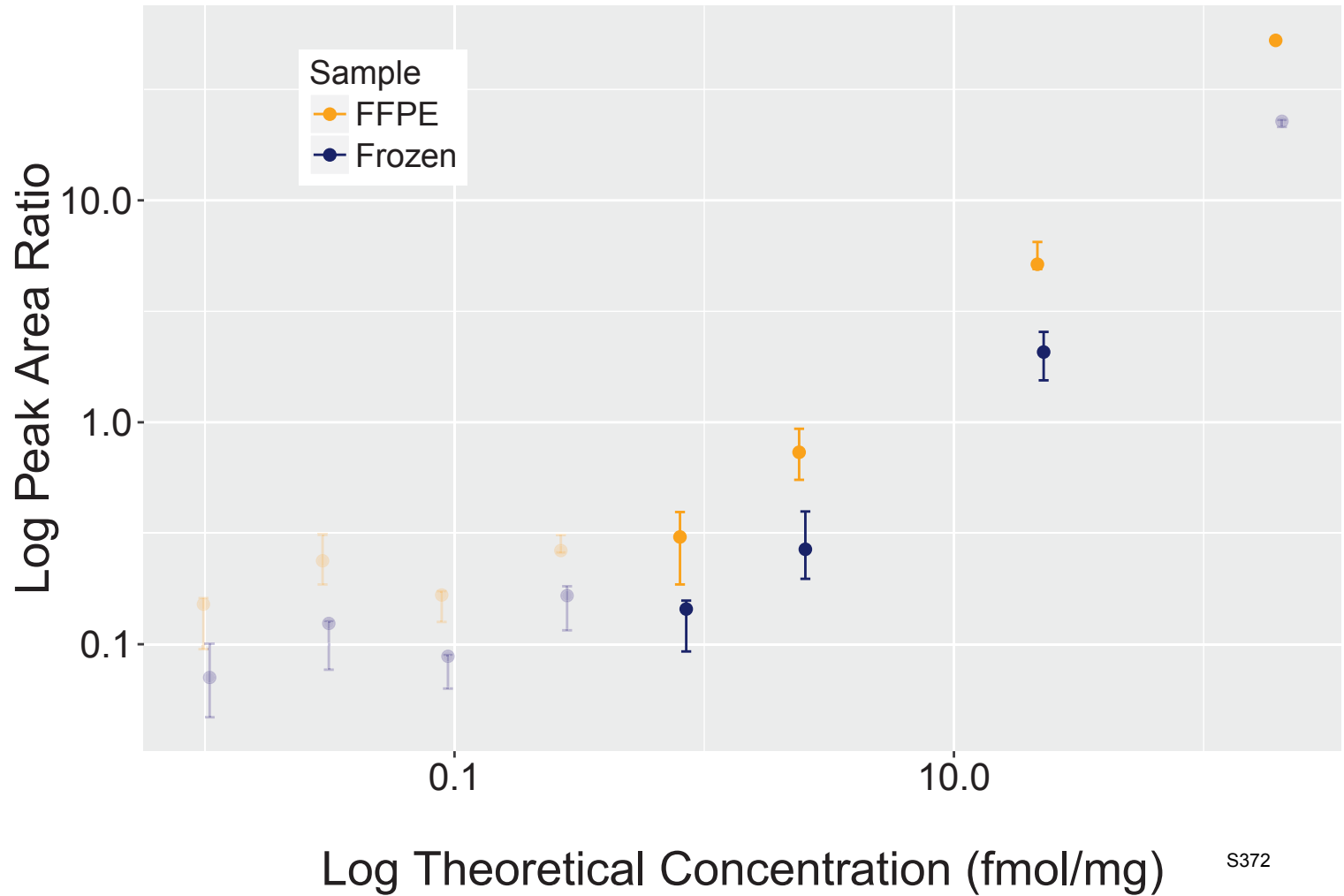
Analyte: CLU.LFDSDPITVTVPEVSR



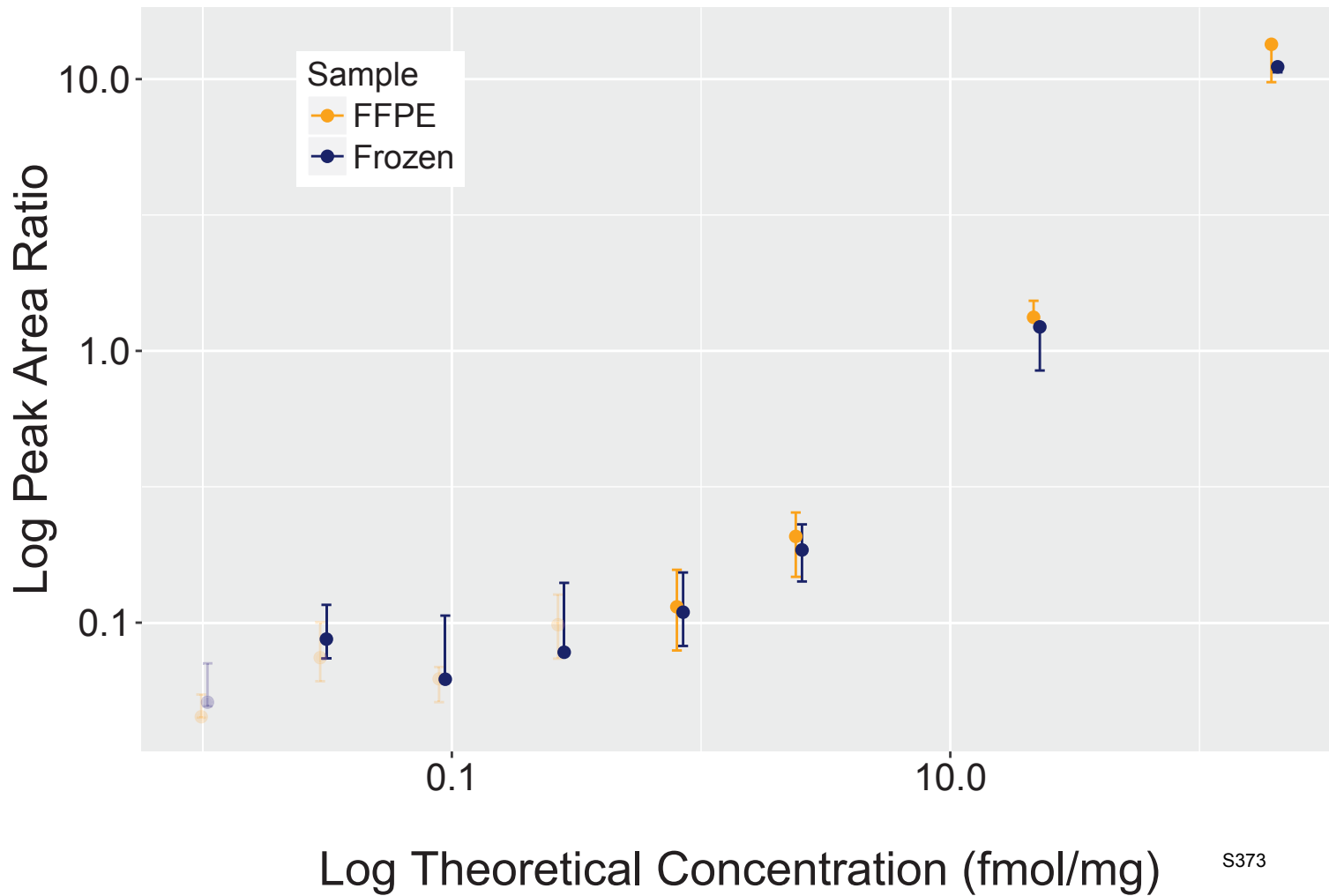
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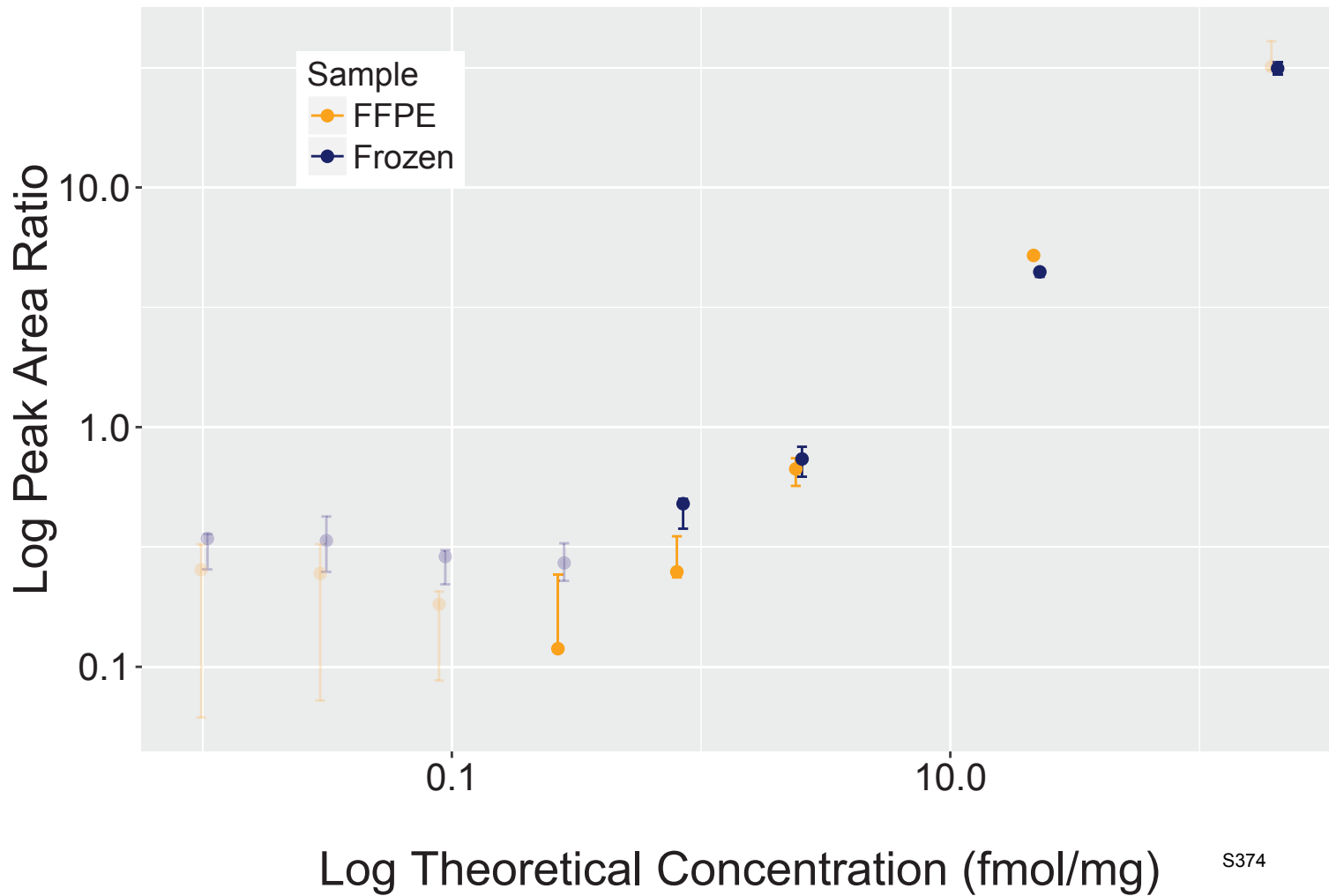
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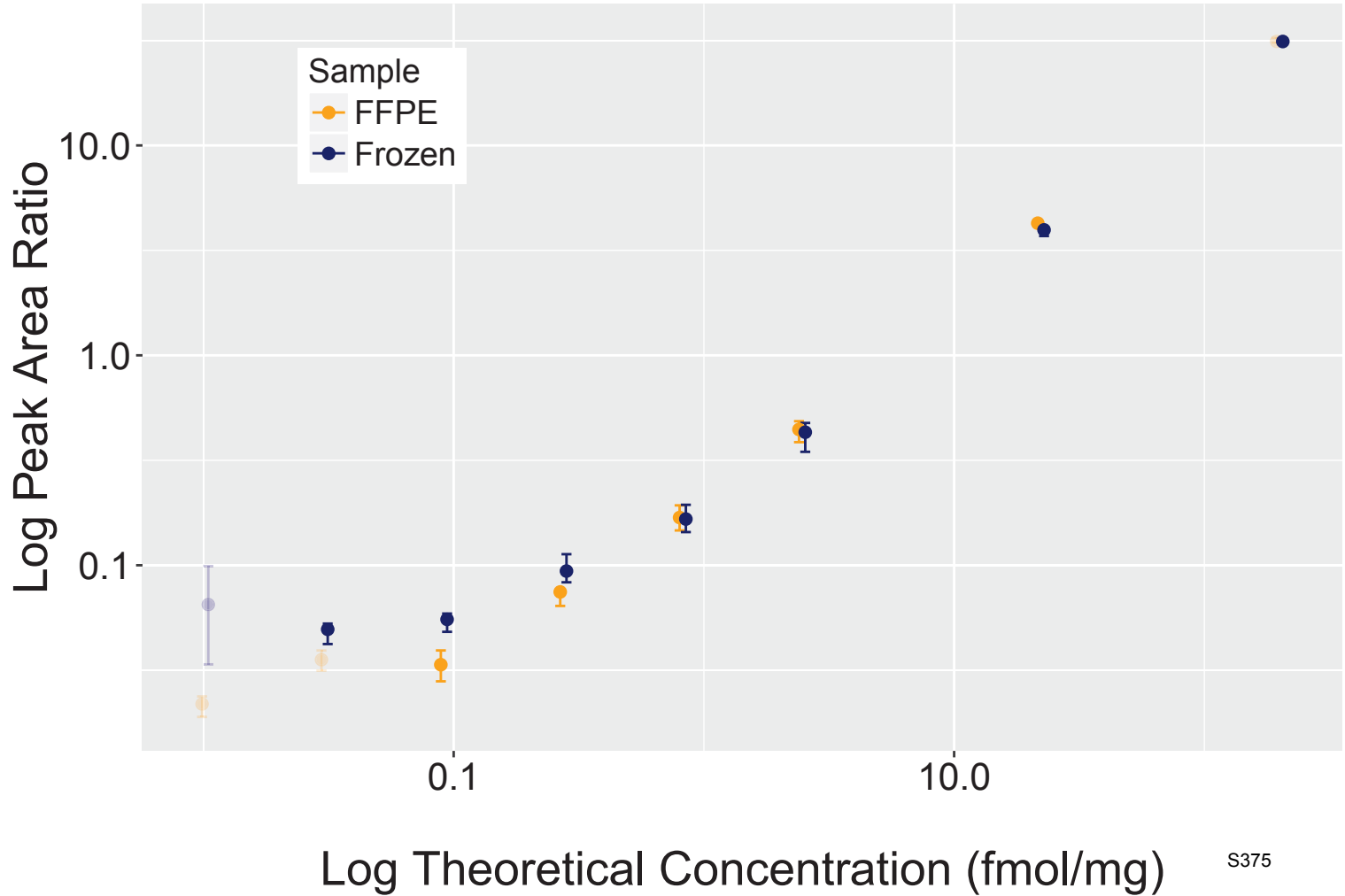
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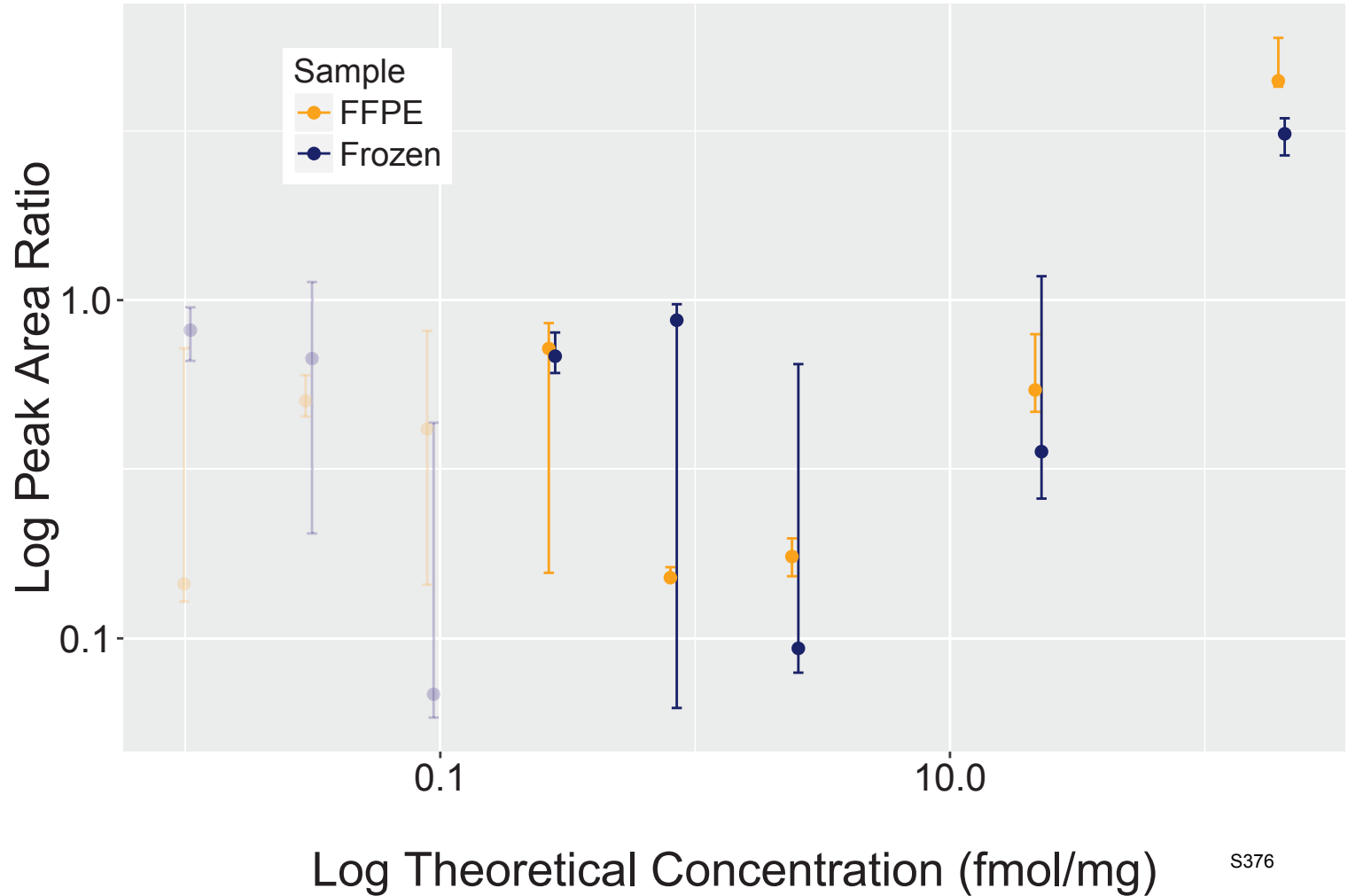
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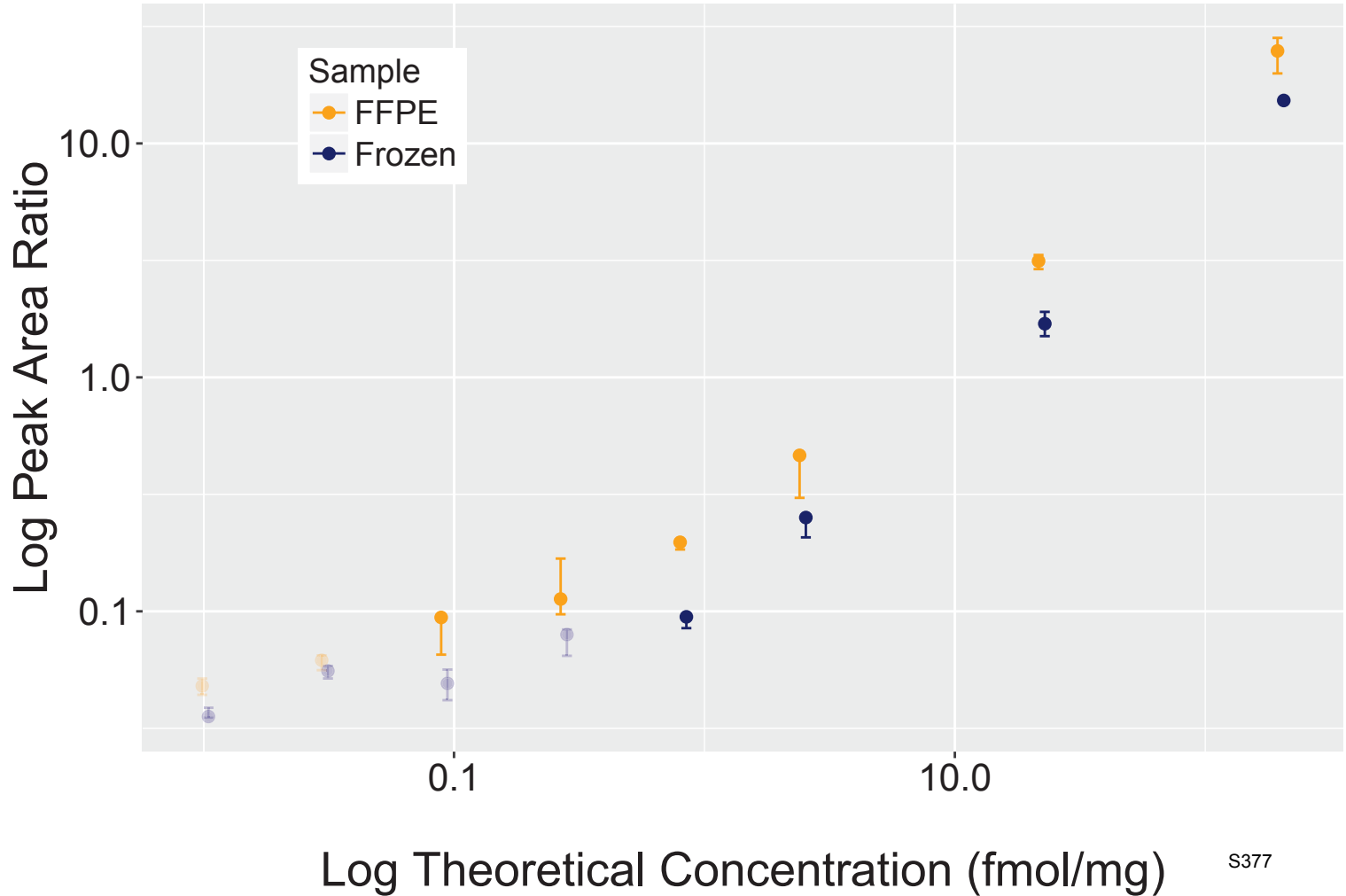
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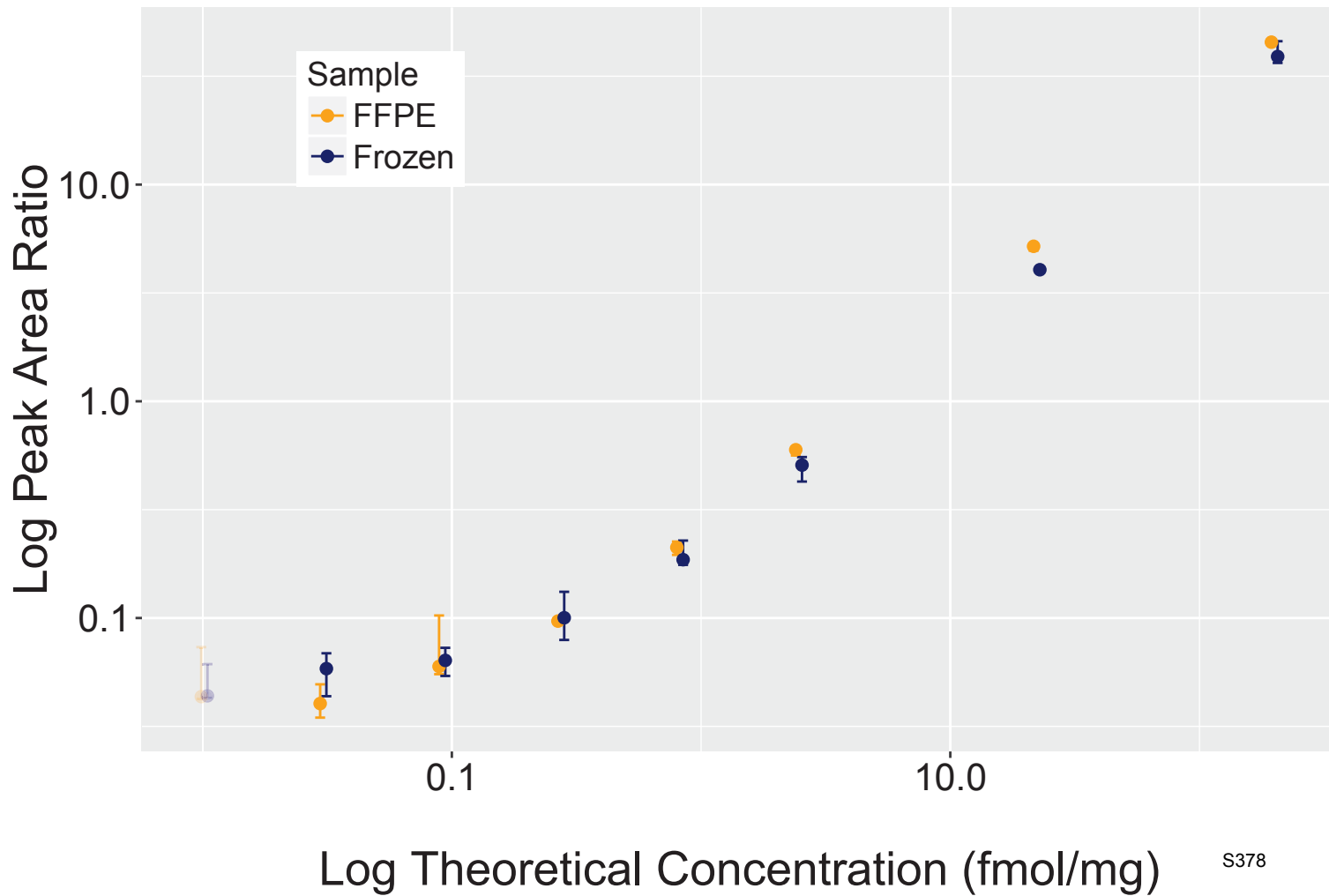
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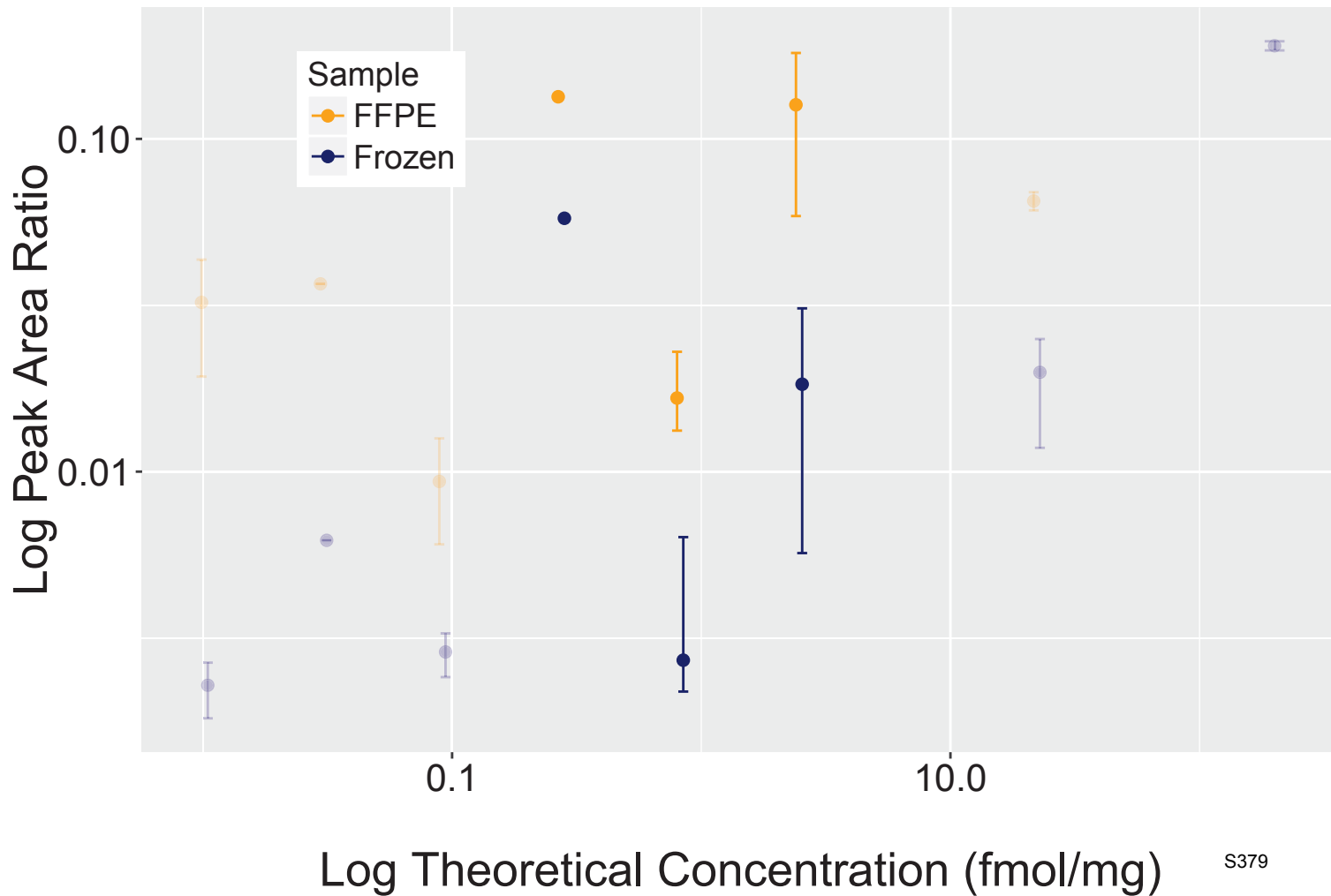
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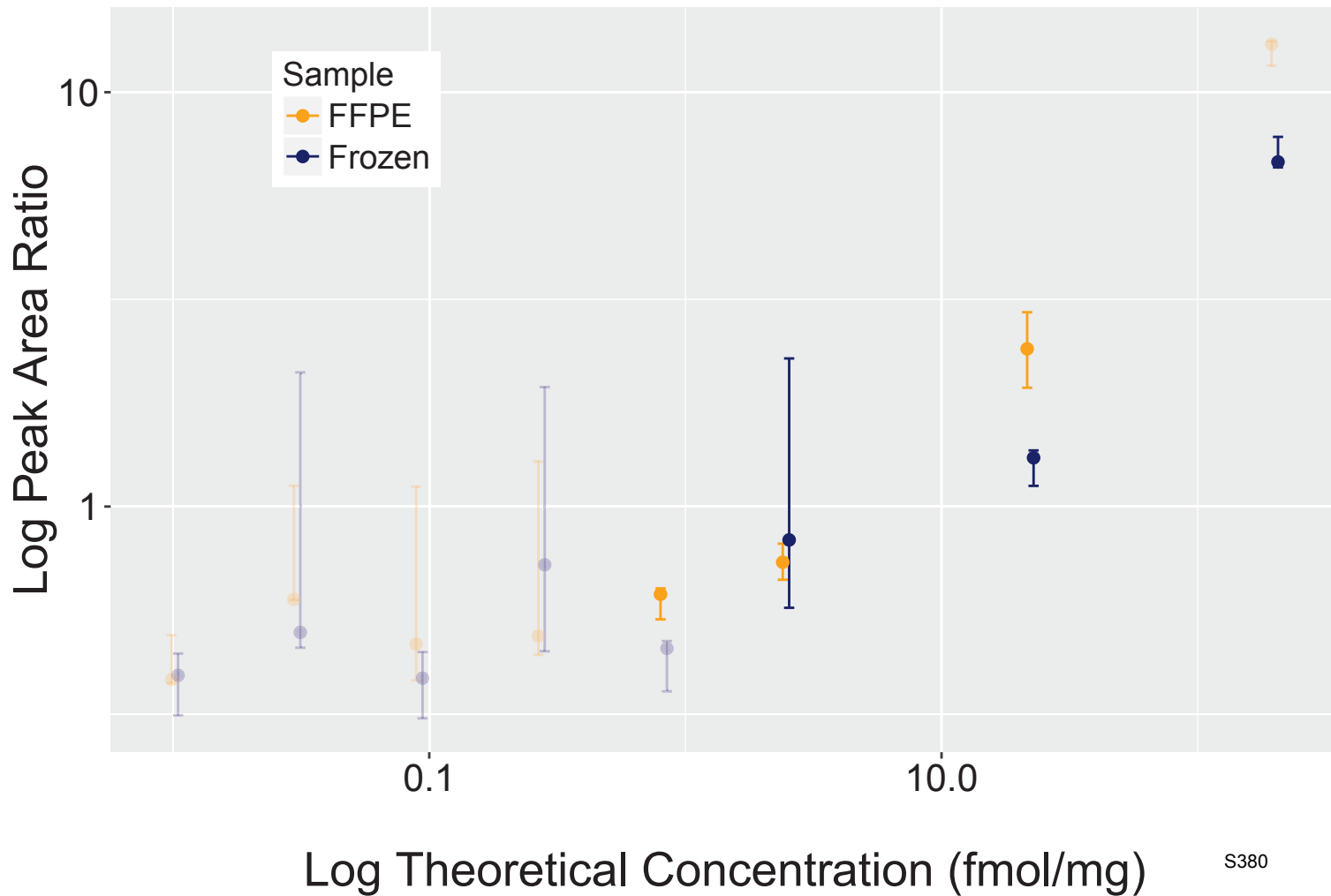
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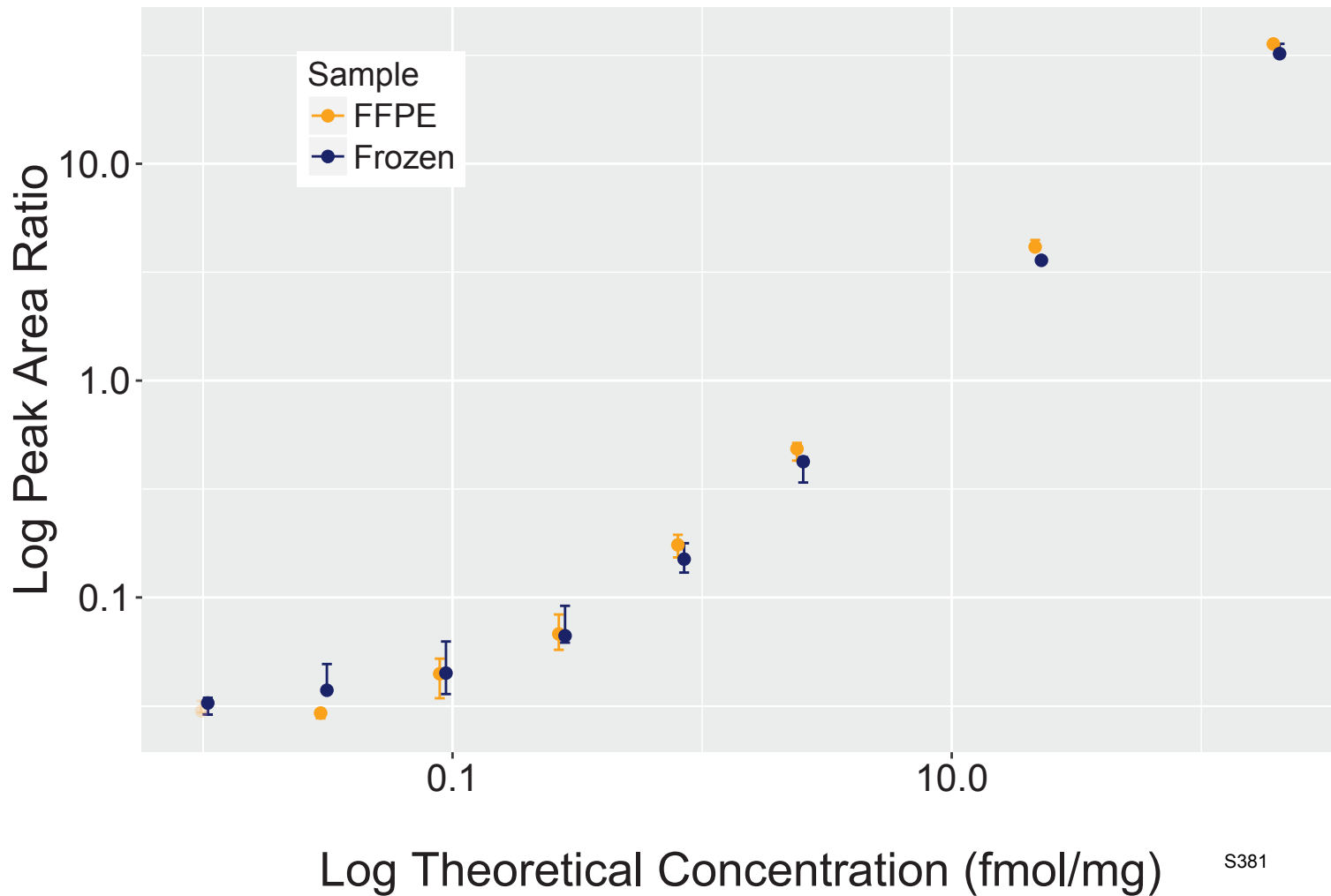
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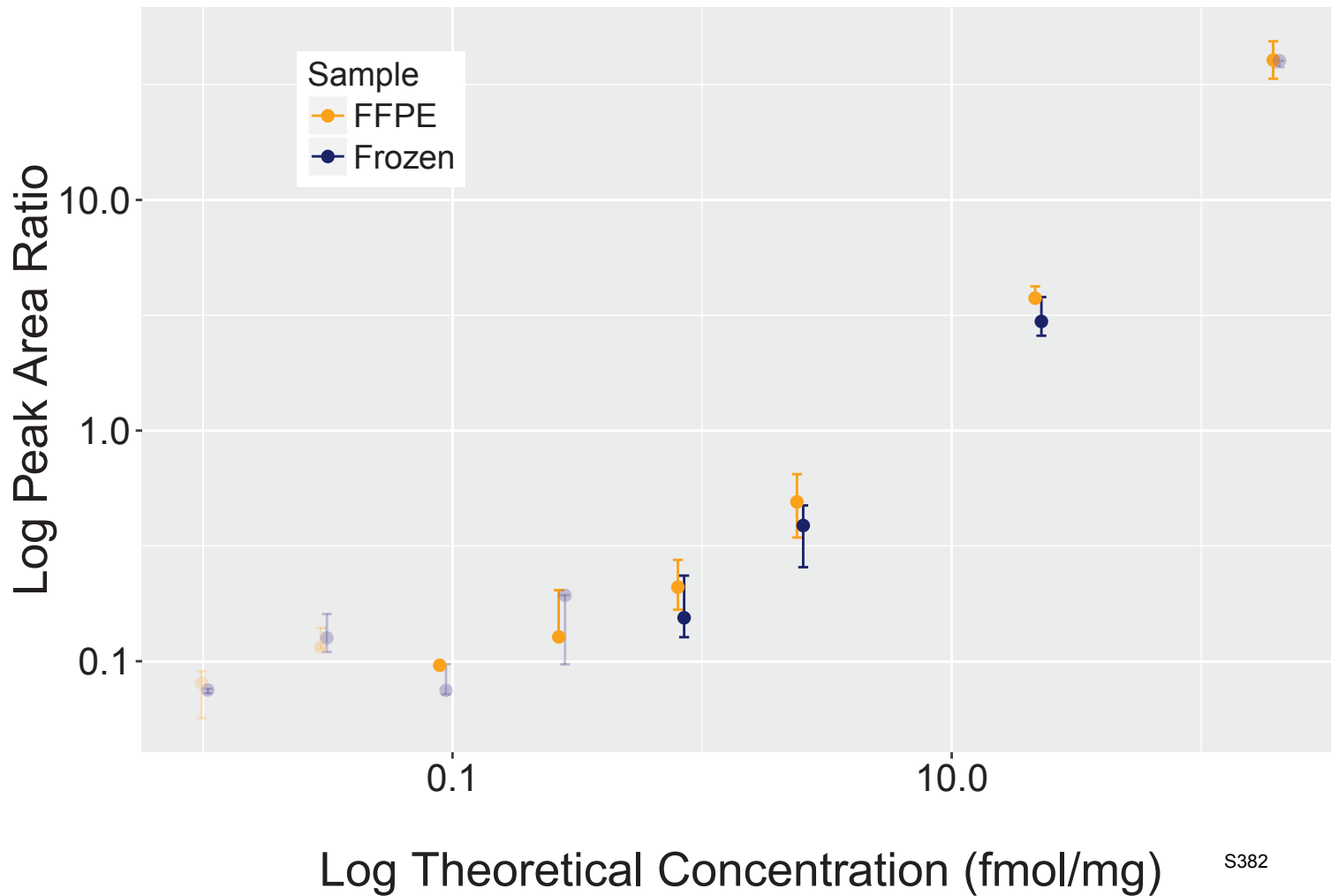
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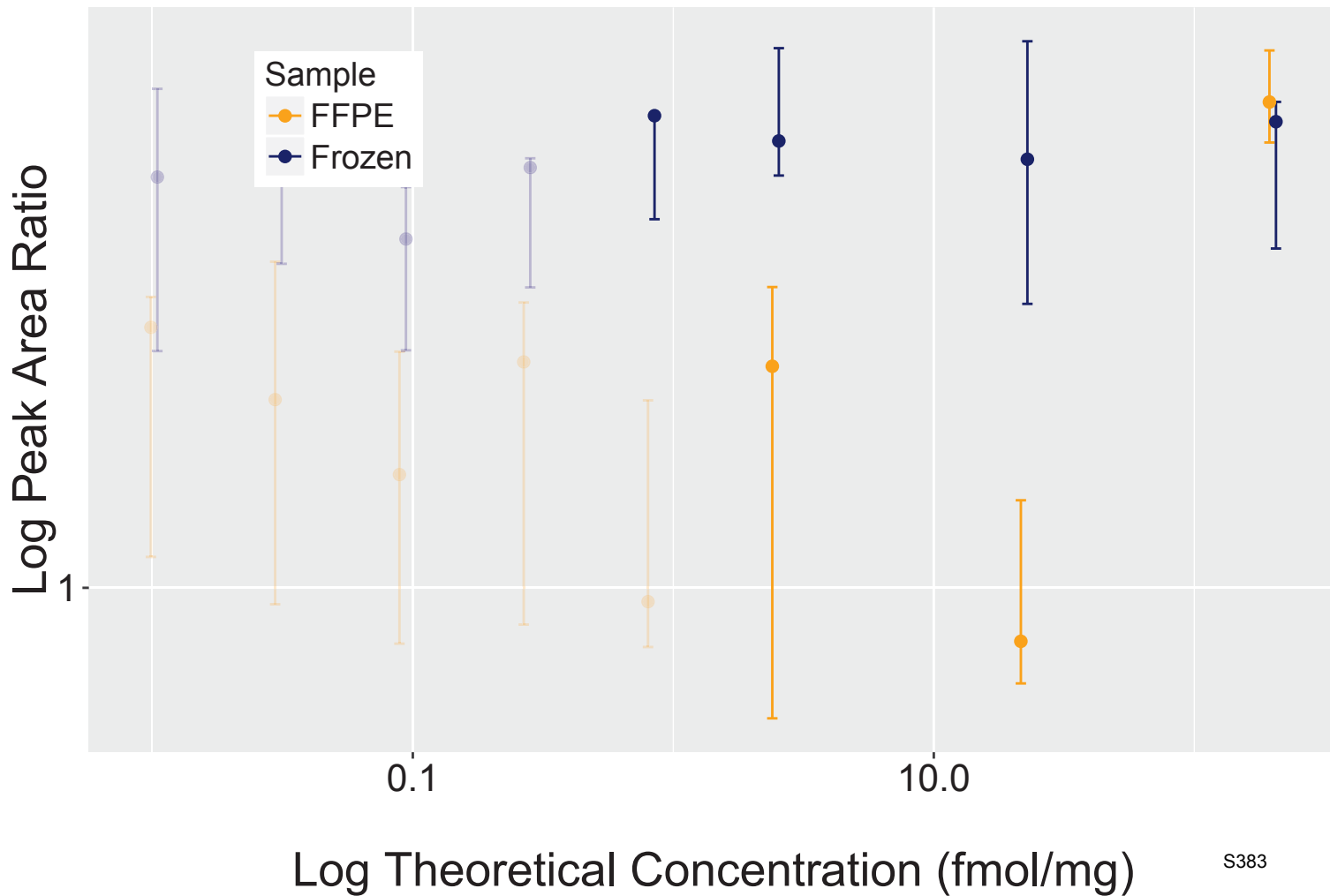
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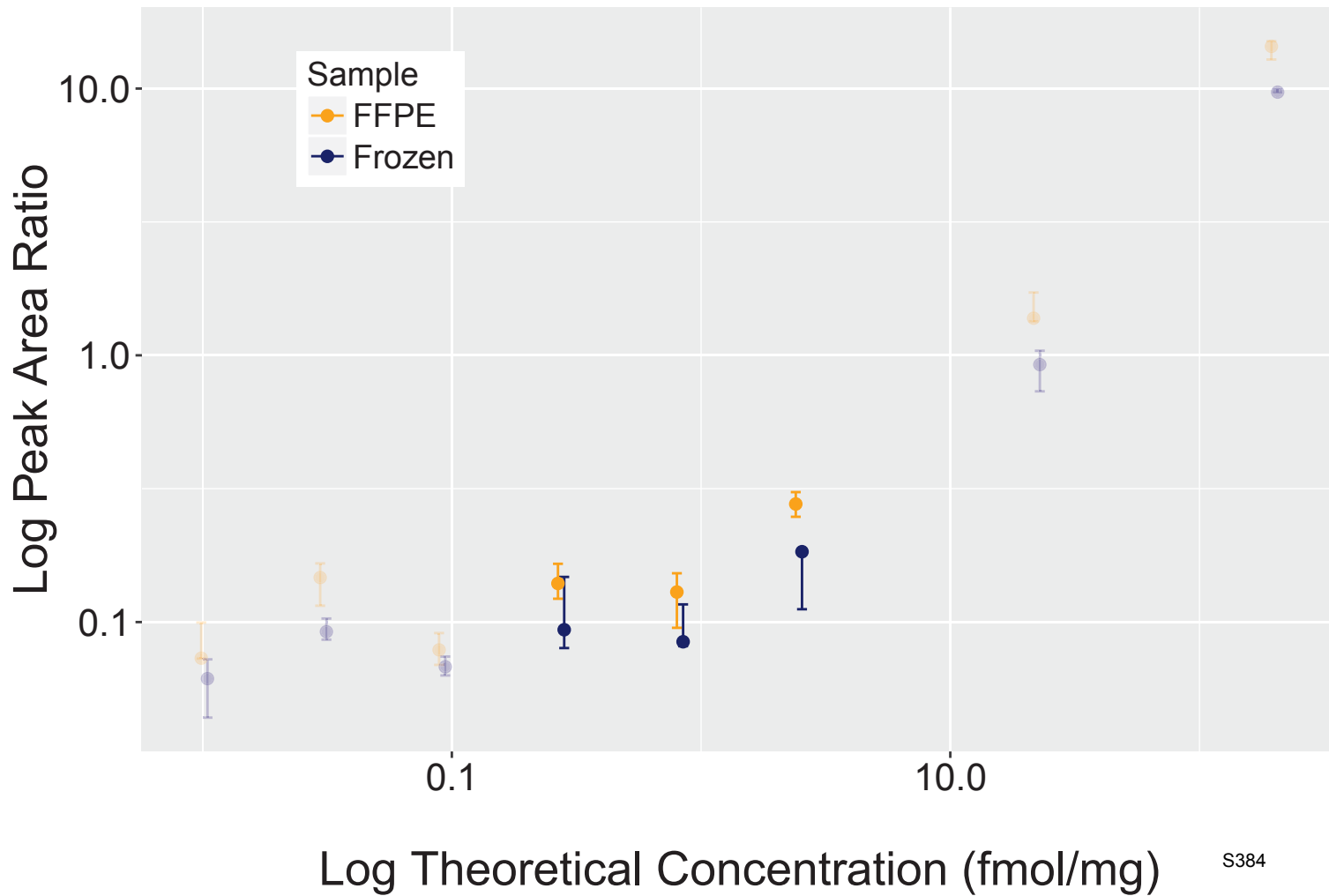
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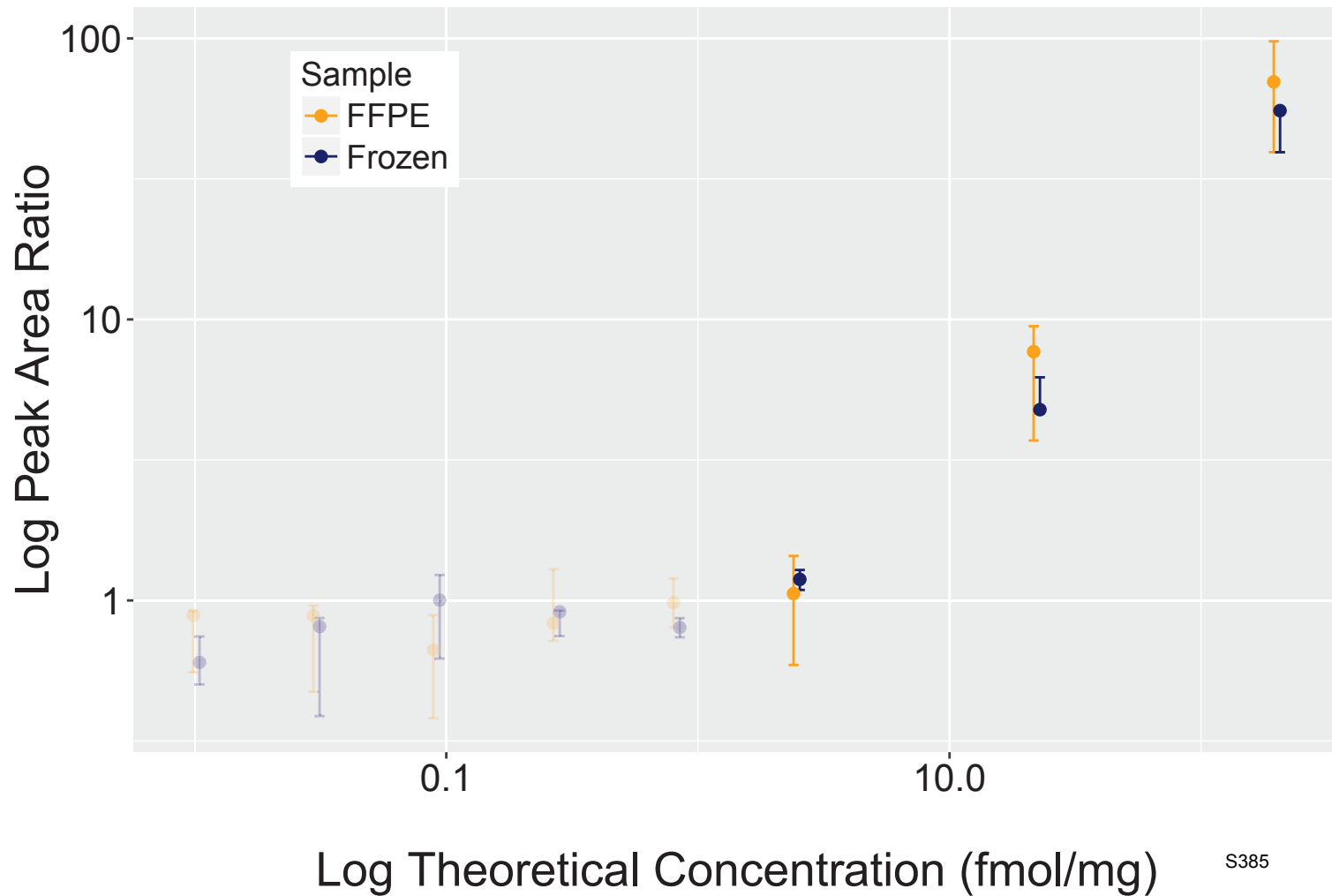
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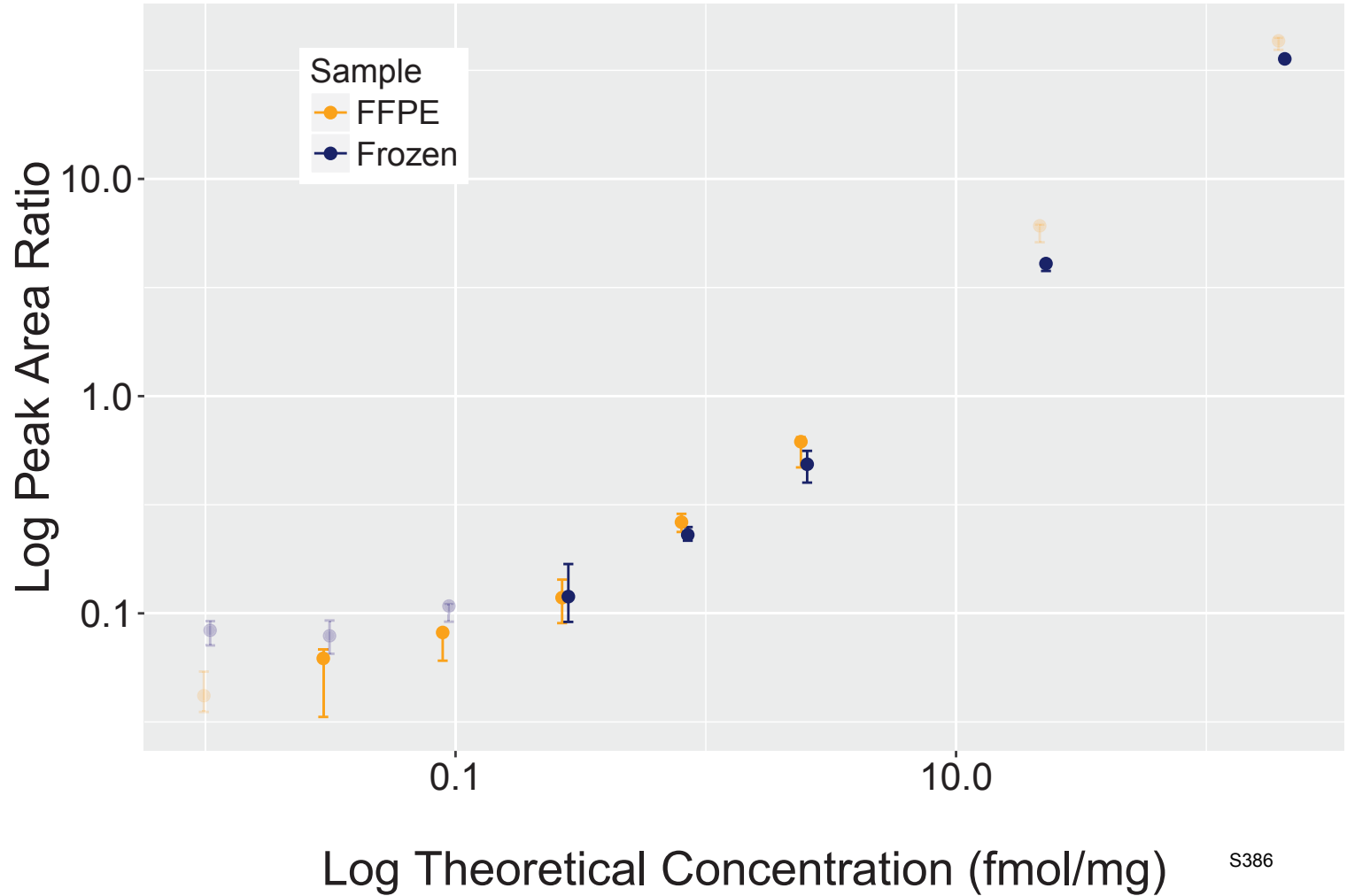
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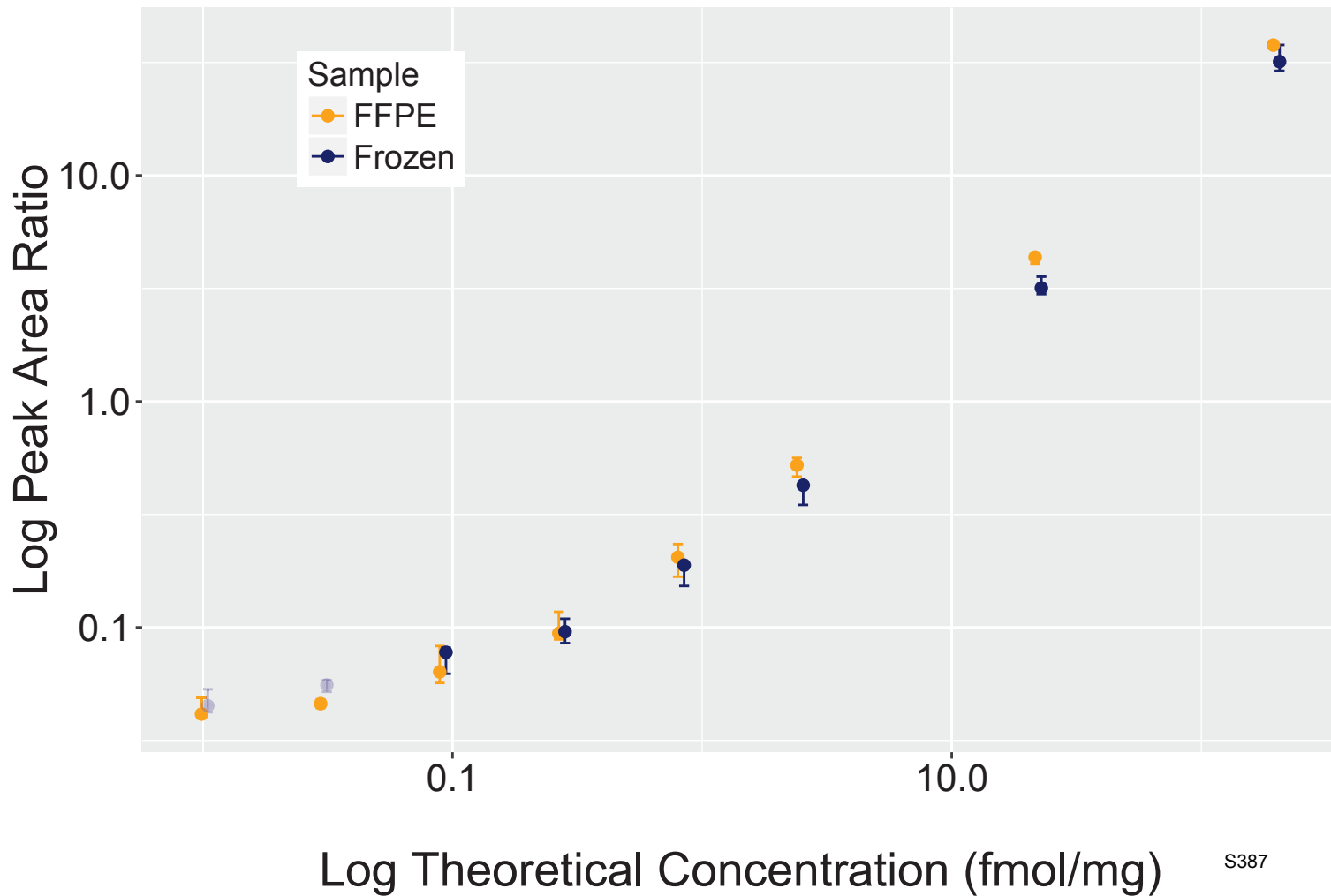
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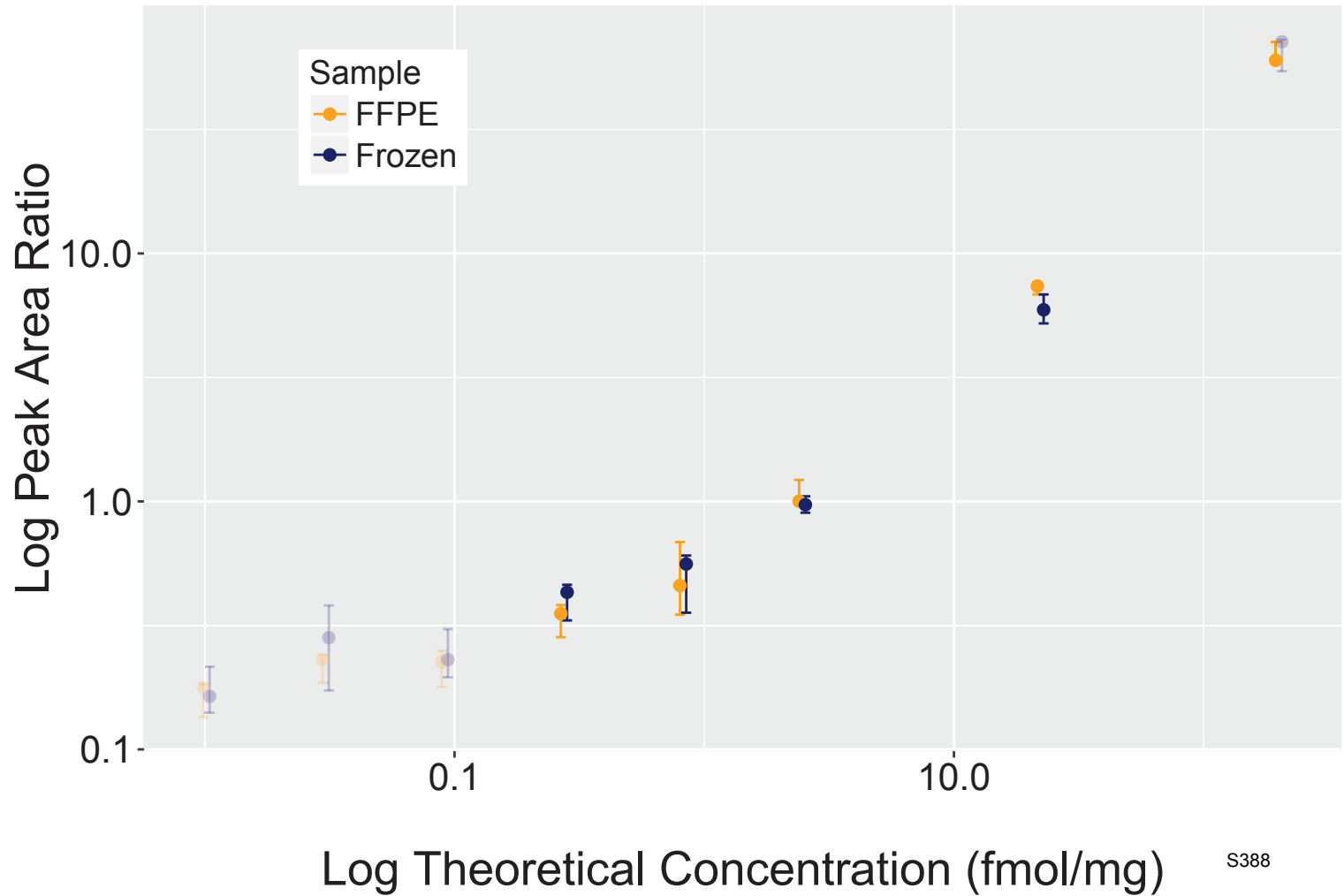
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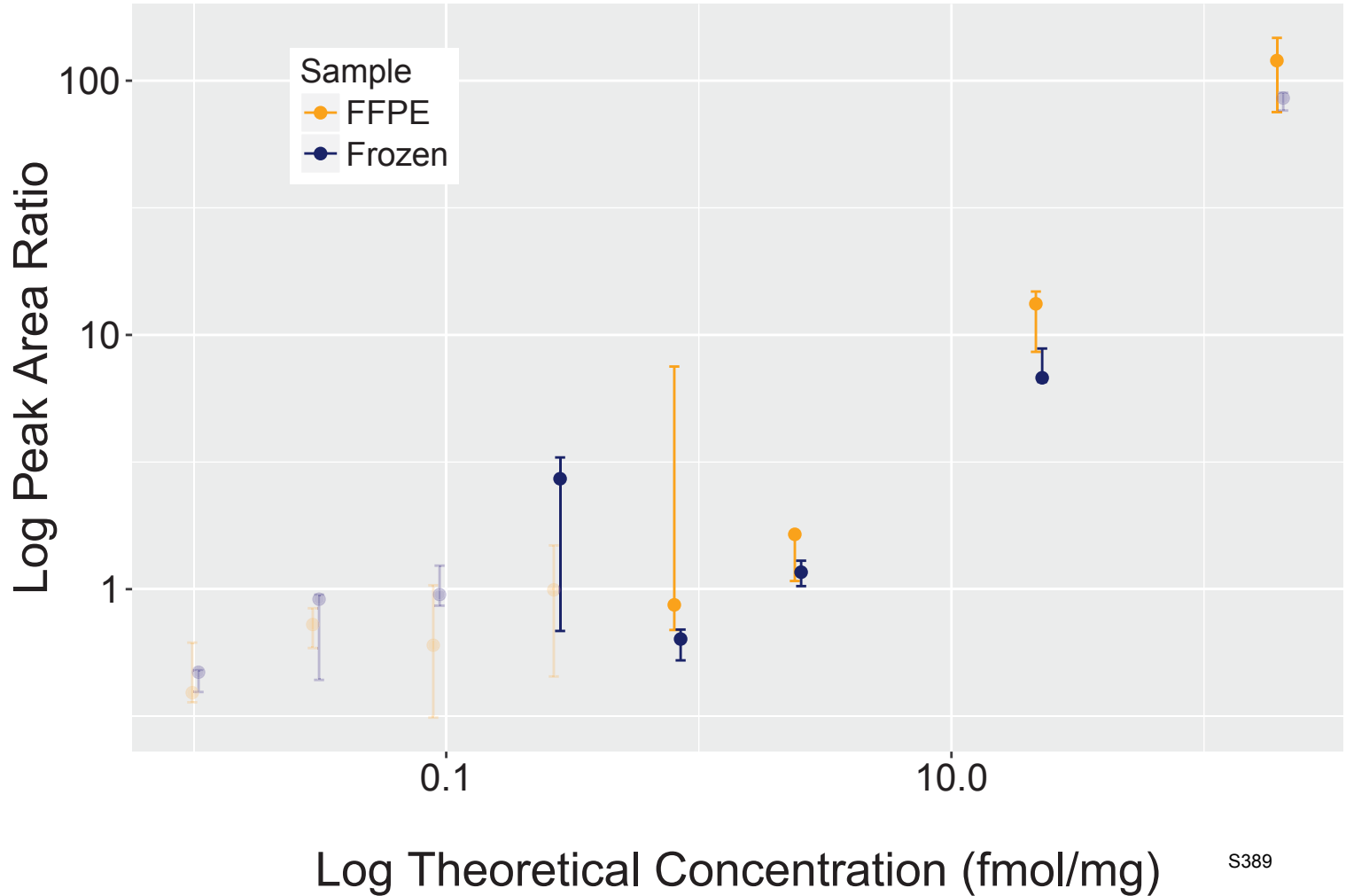
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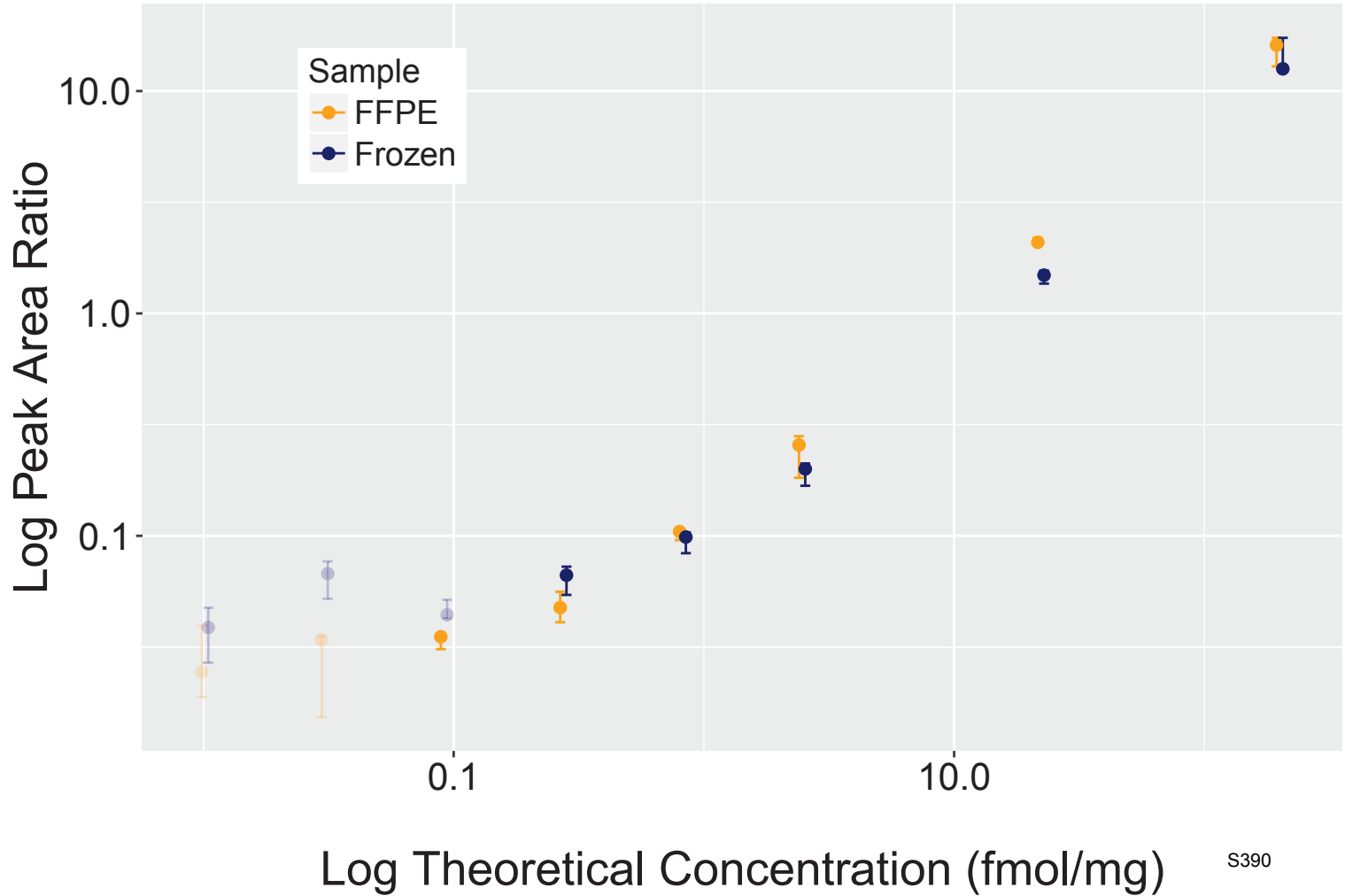
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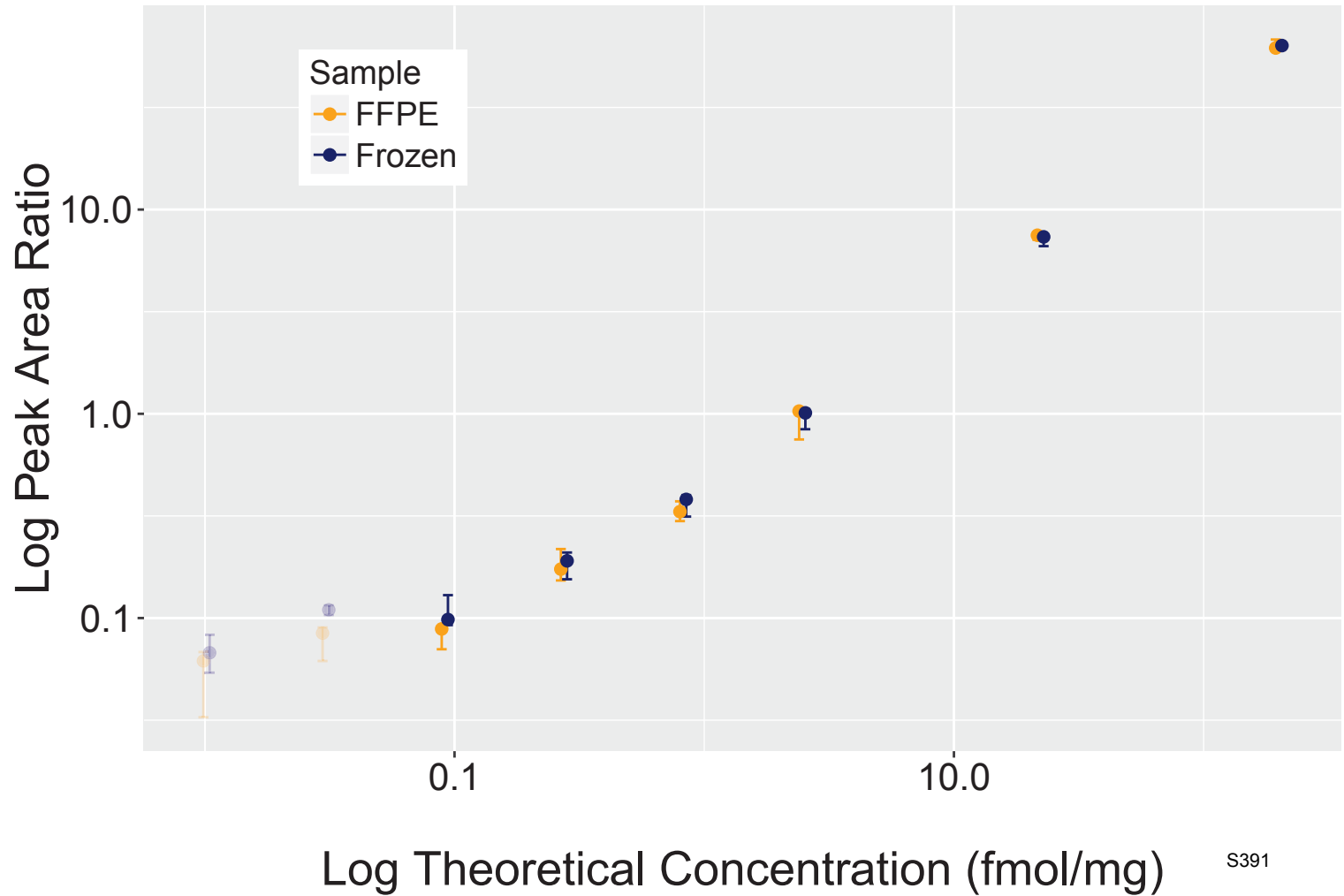
Analyte: GRB7.LIGQQGLVDGLFLVR



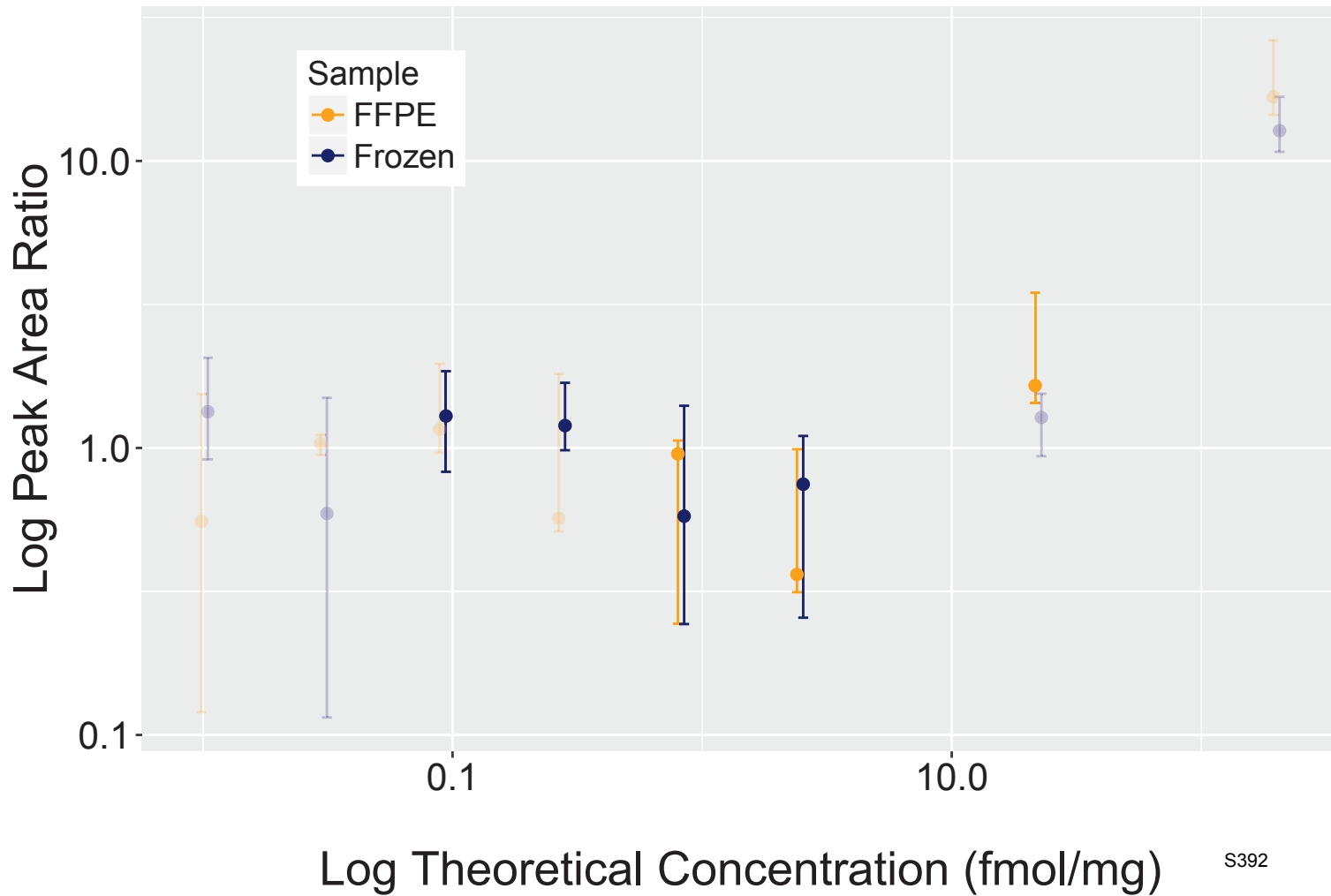
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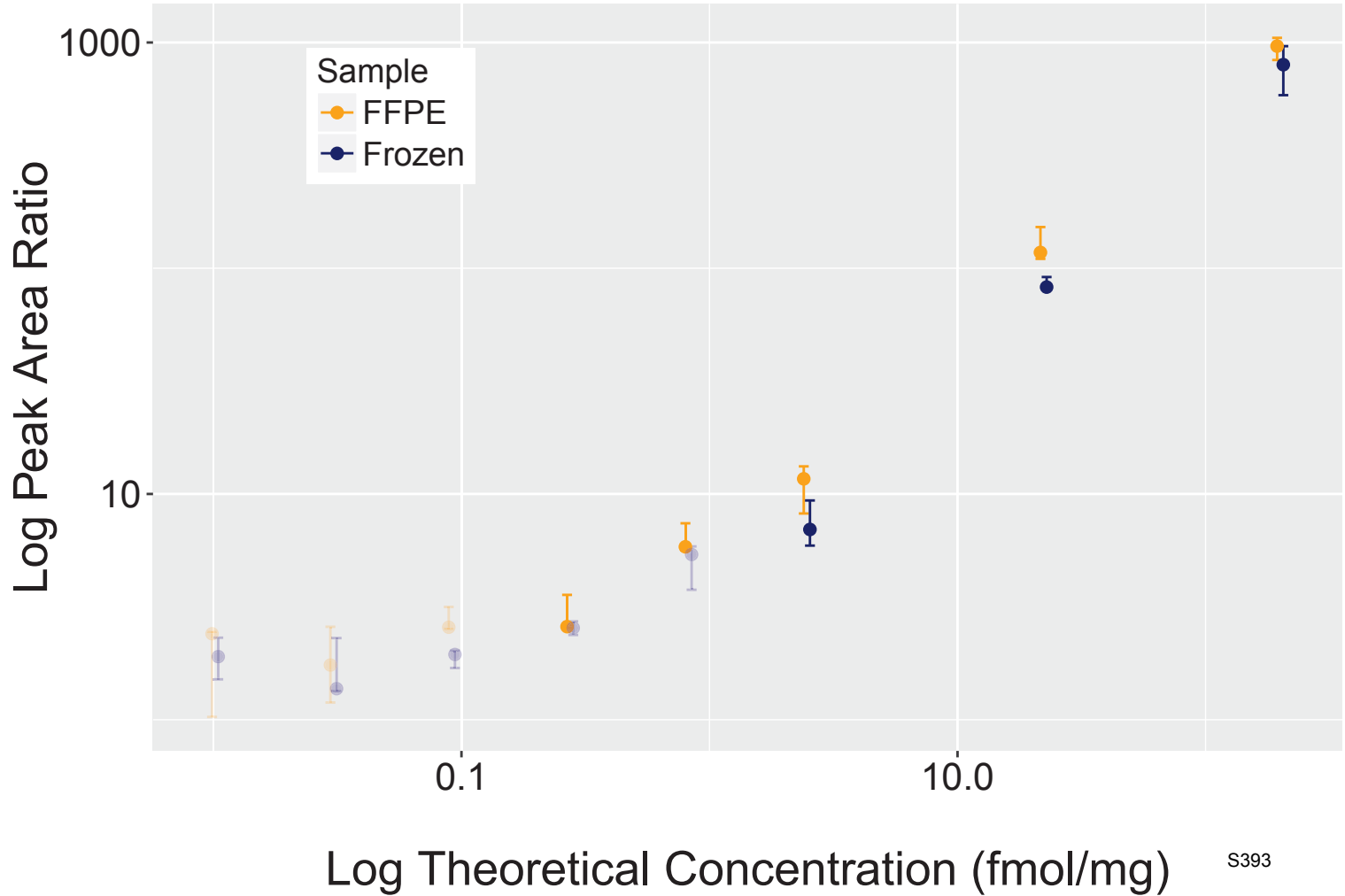
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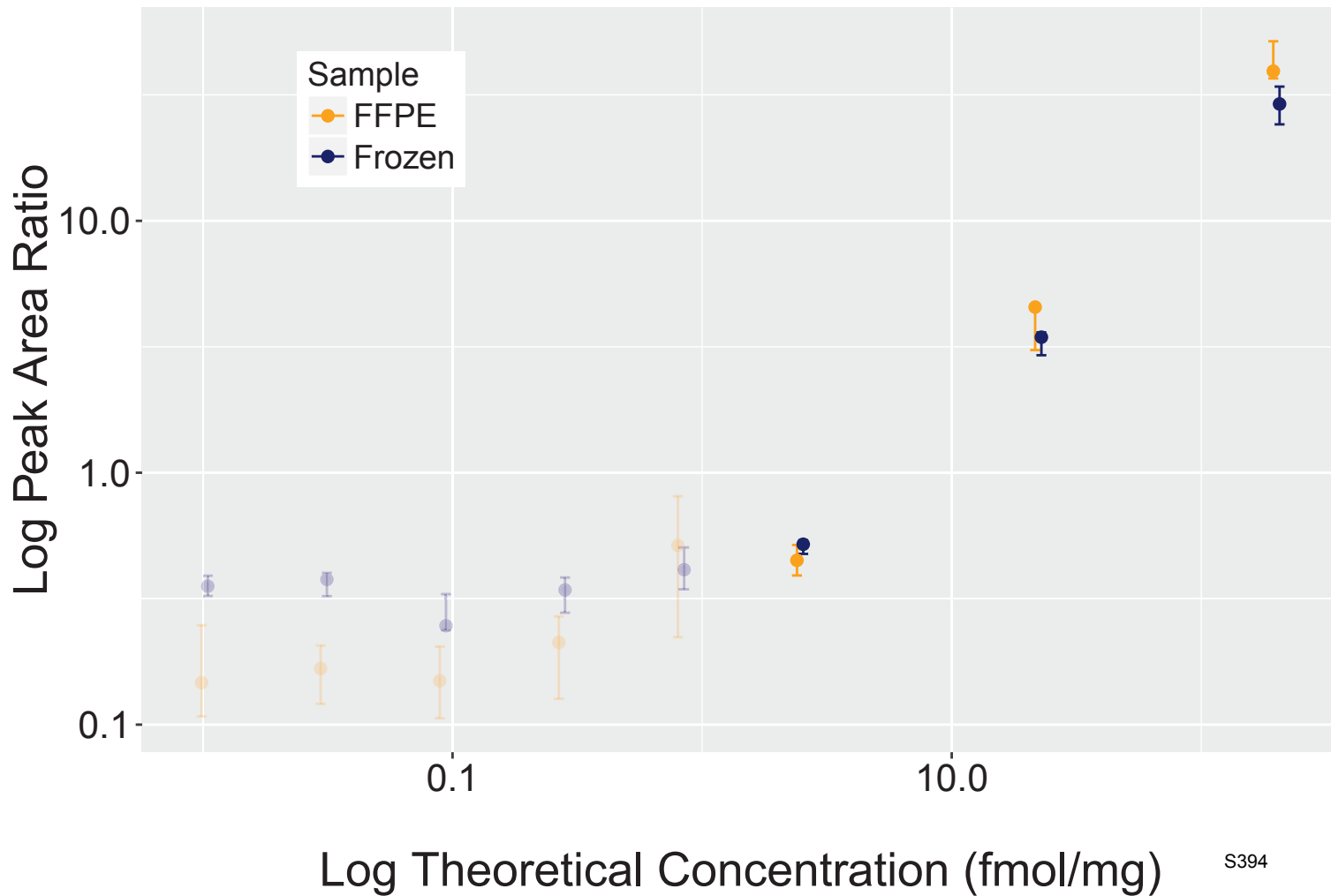
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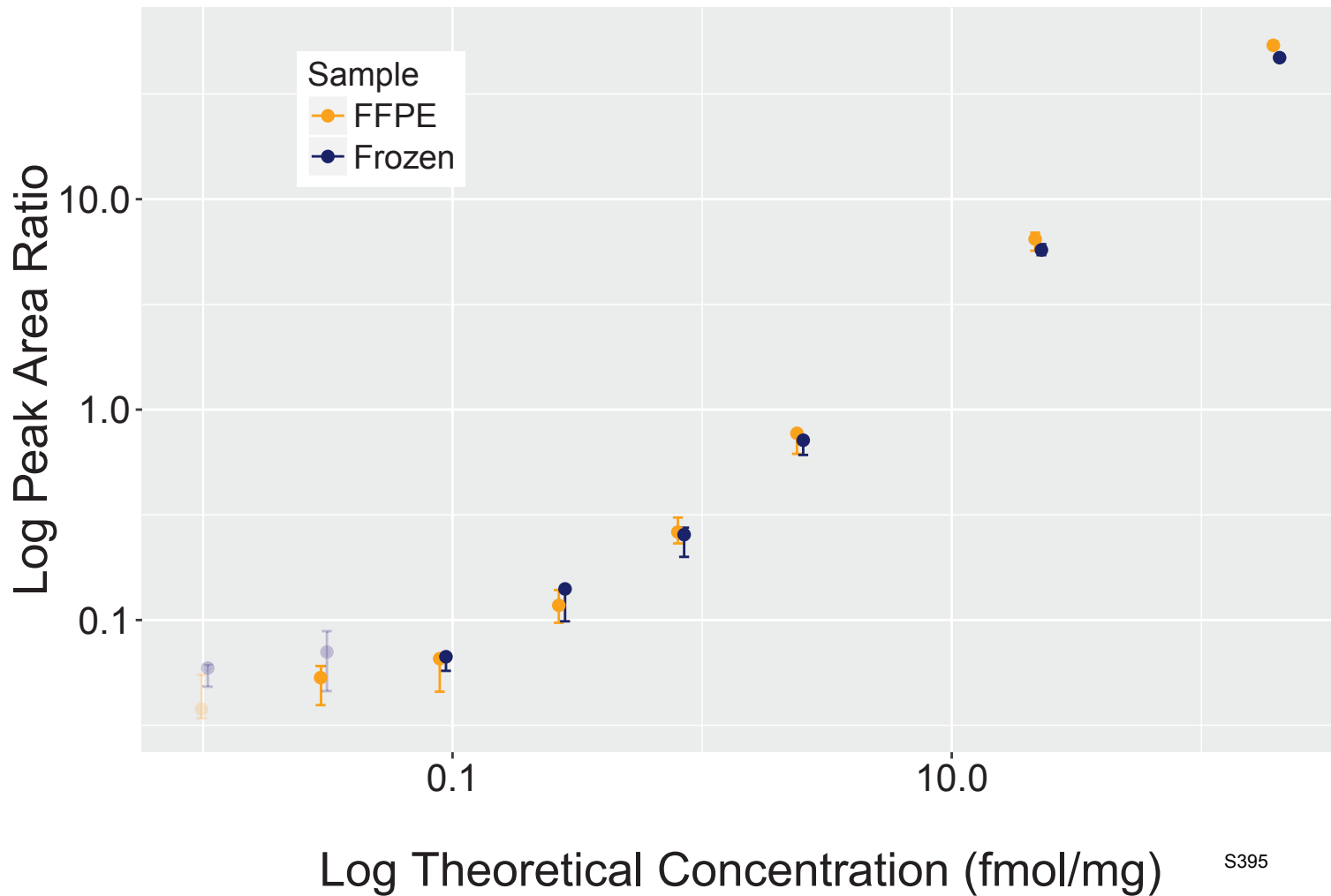
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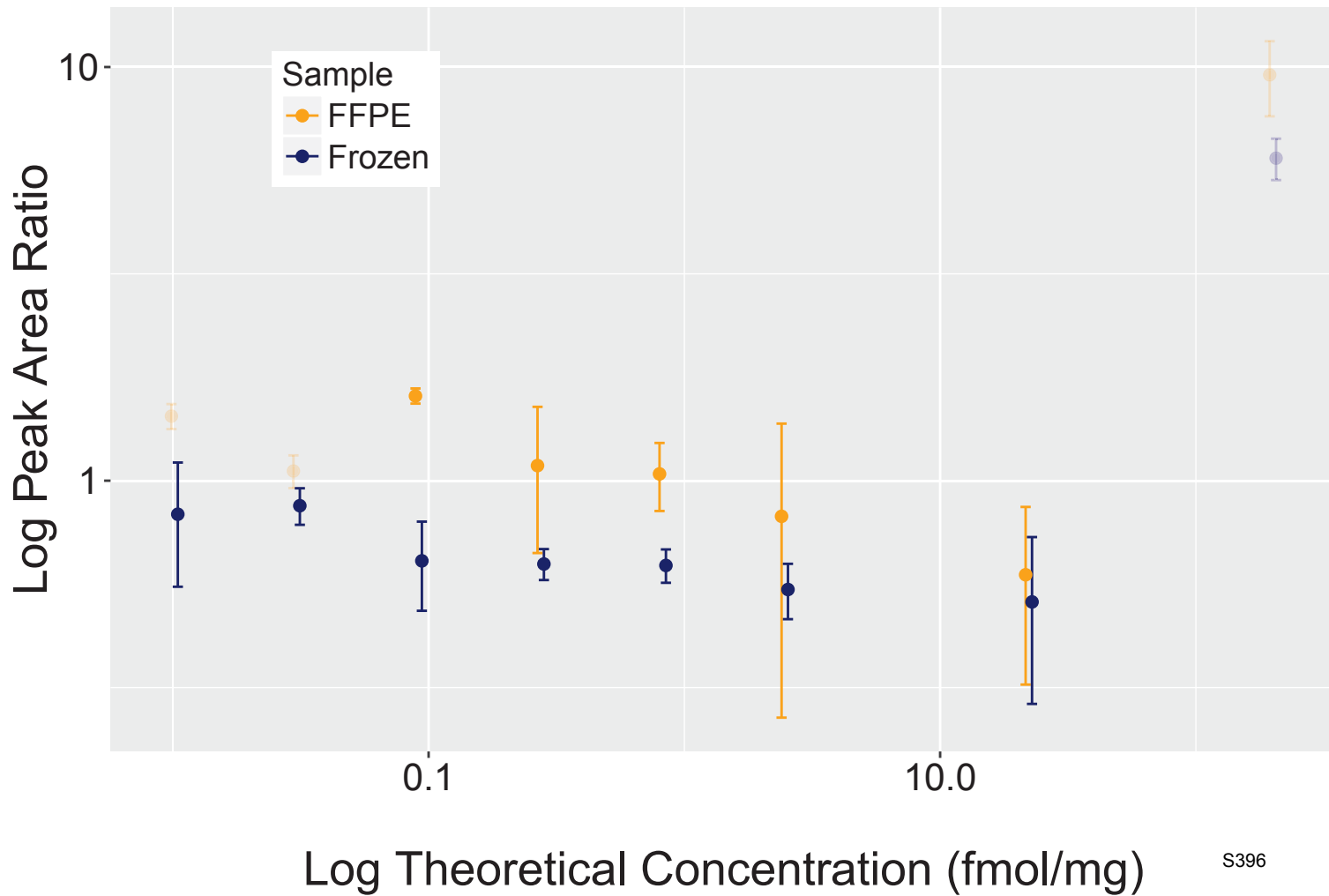
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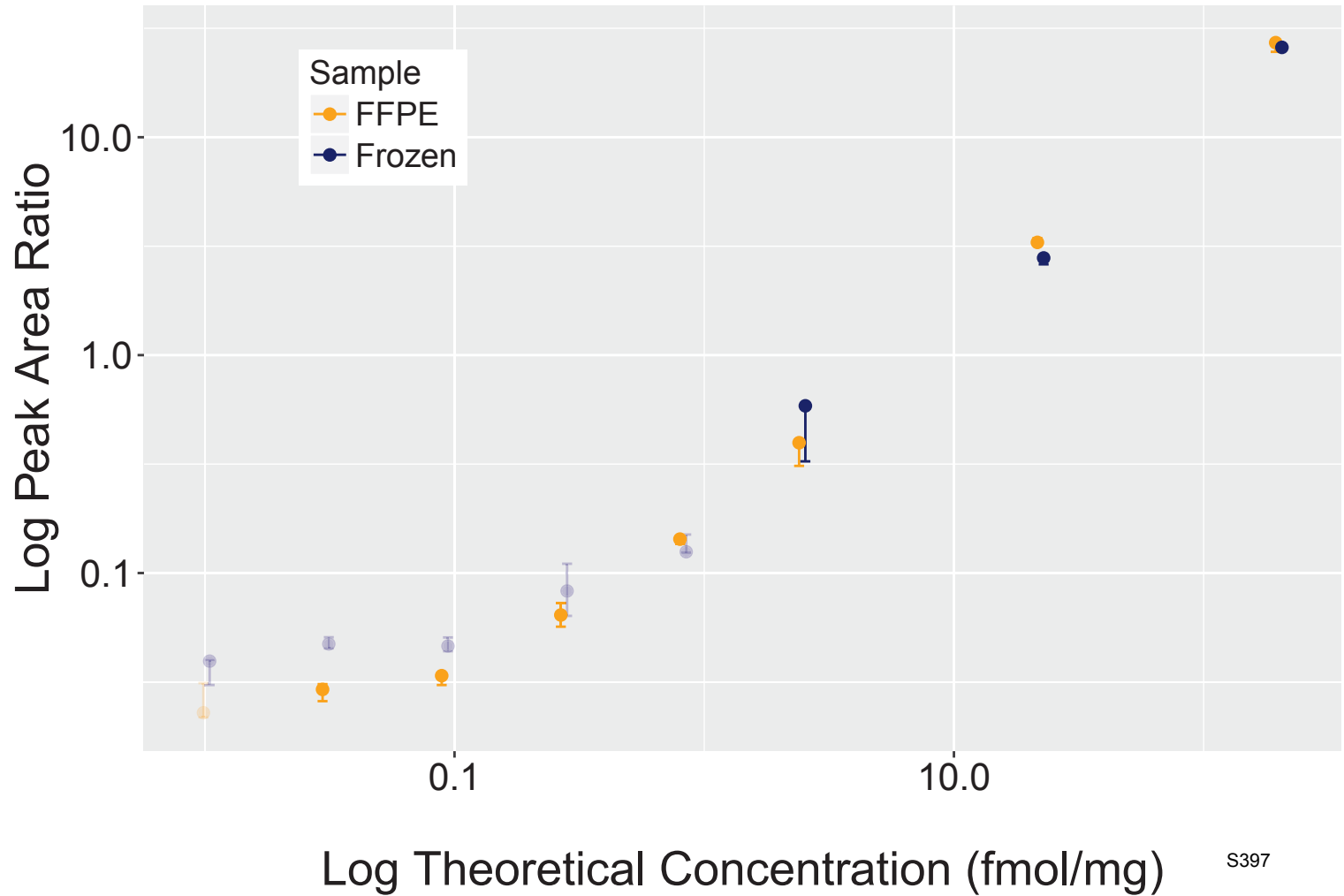
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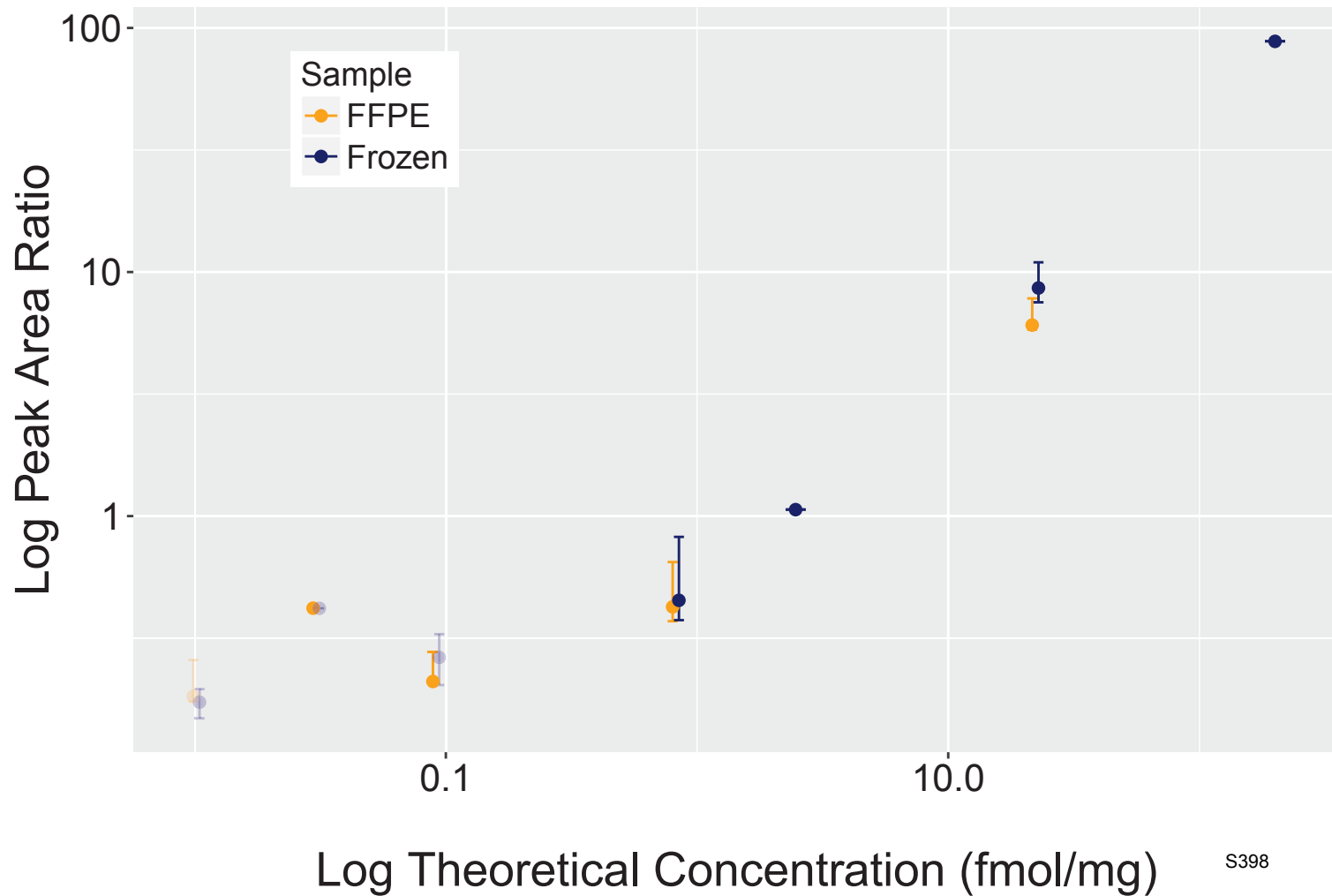
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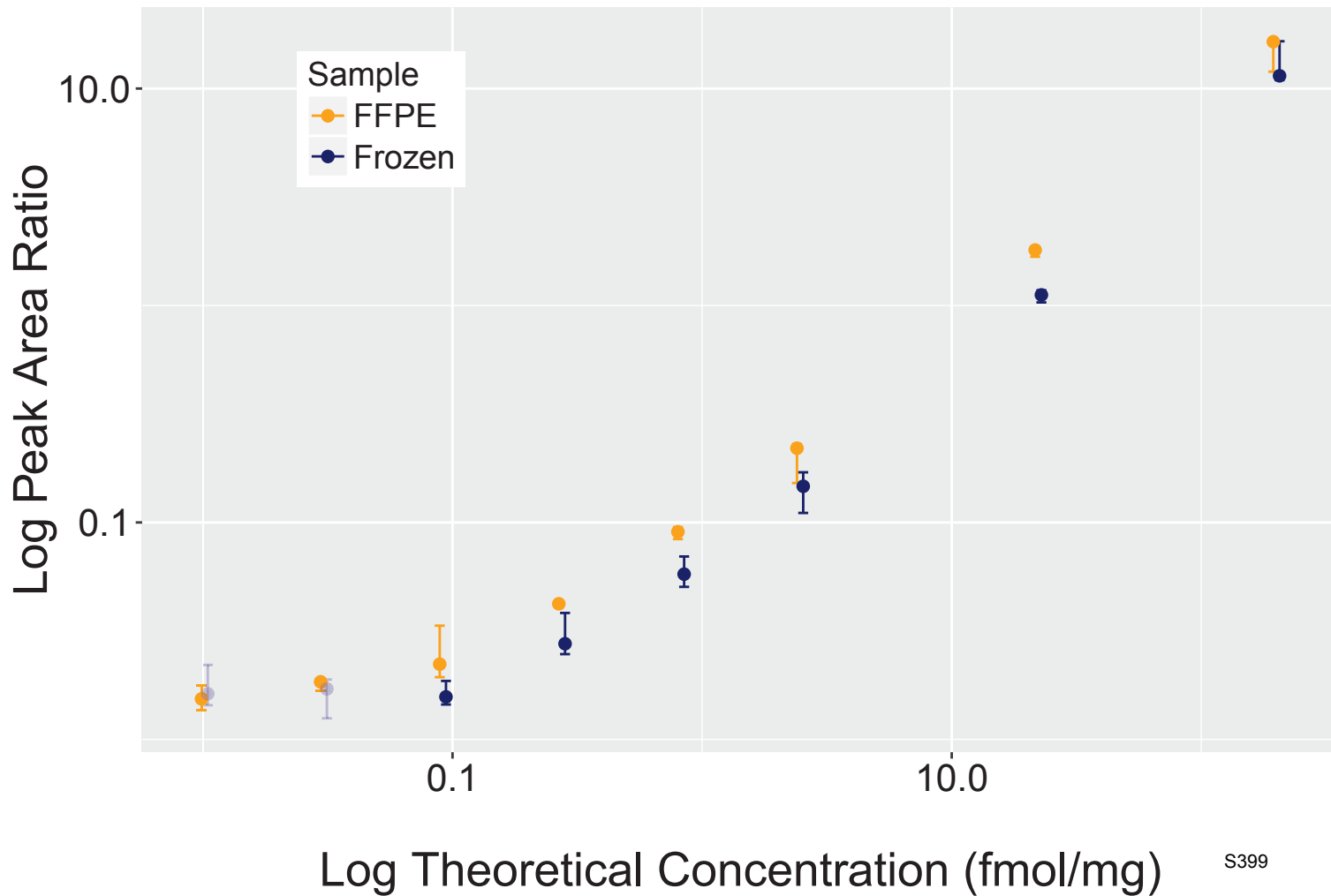
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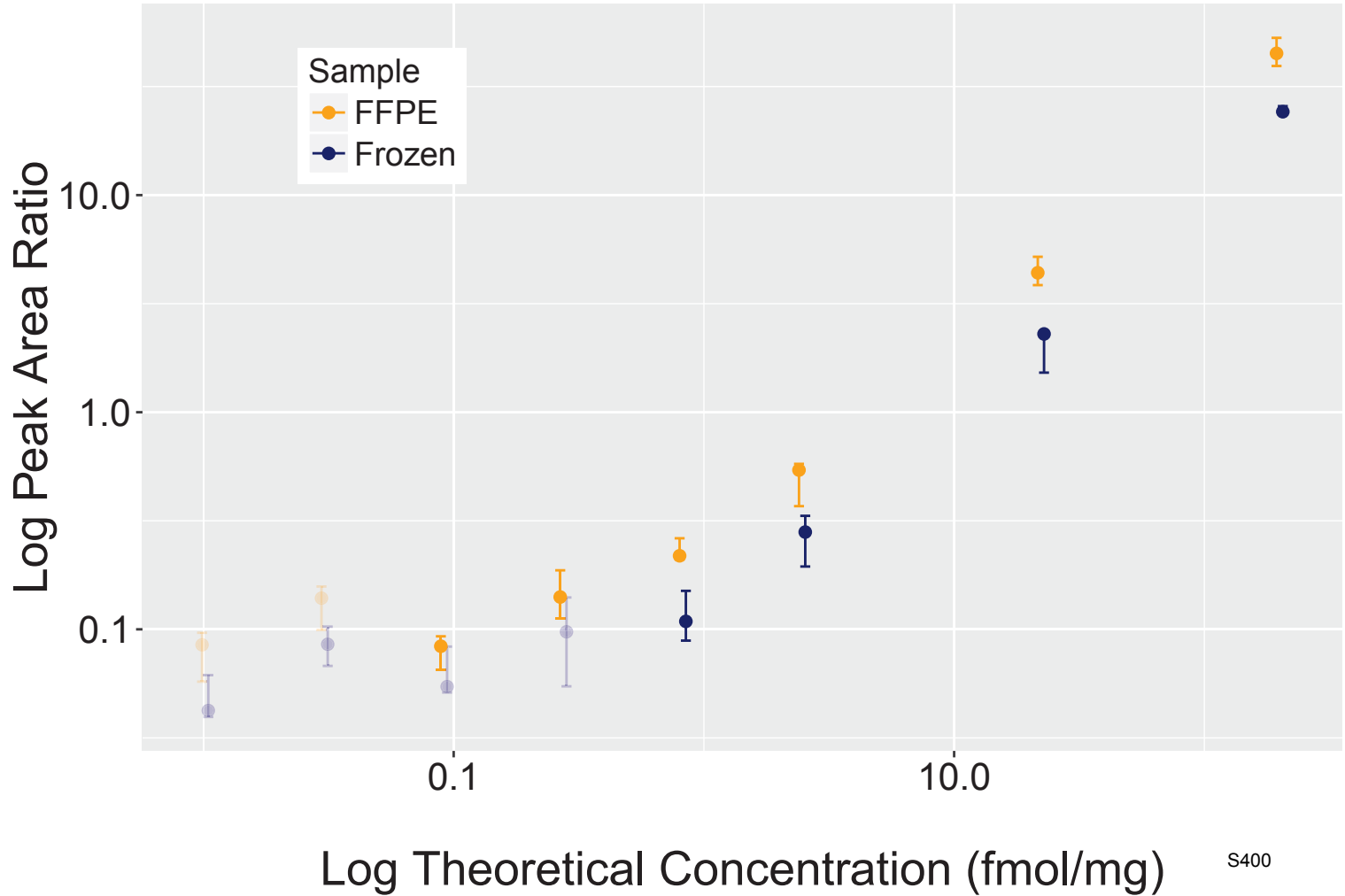
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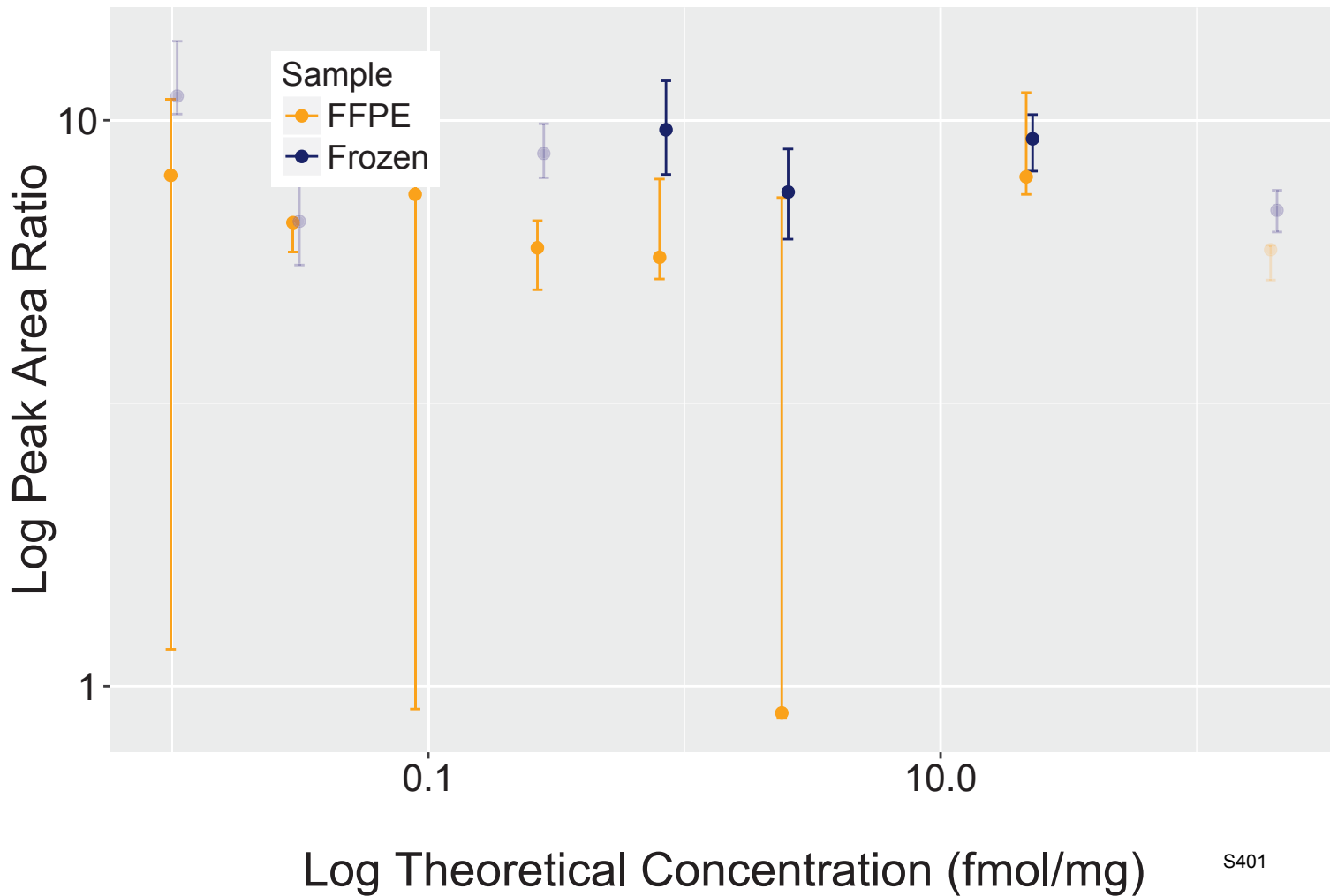
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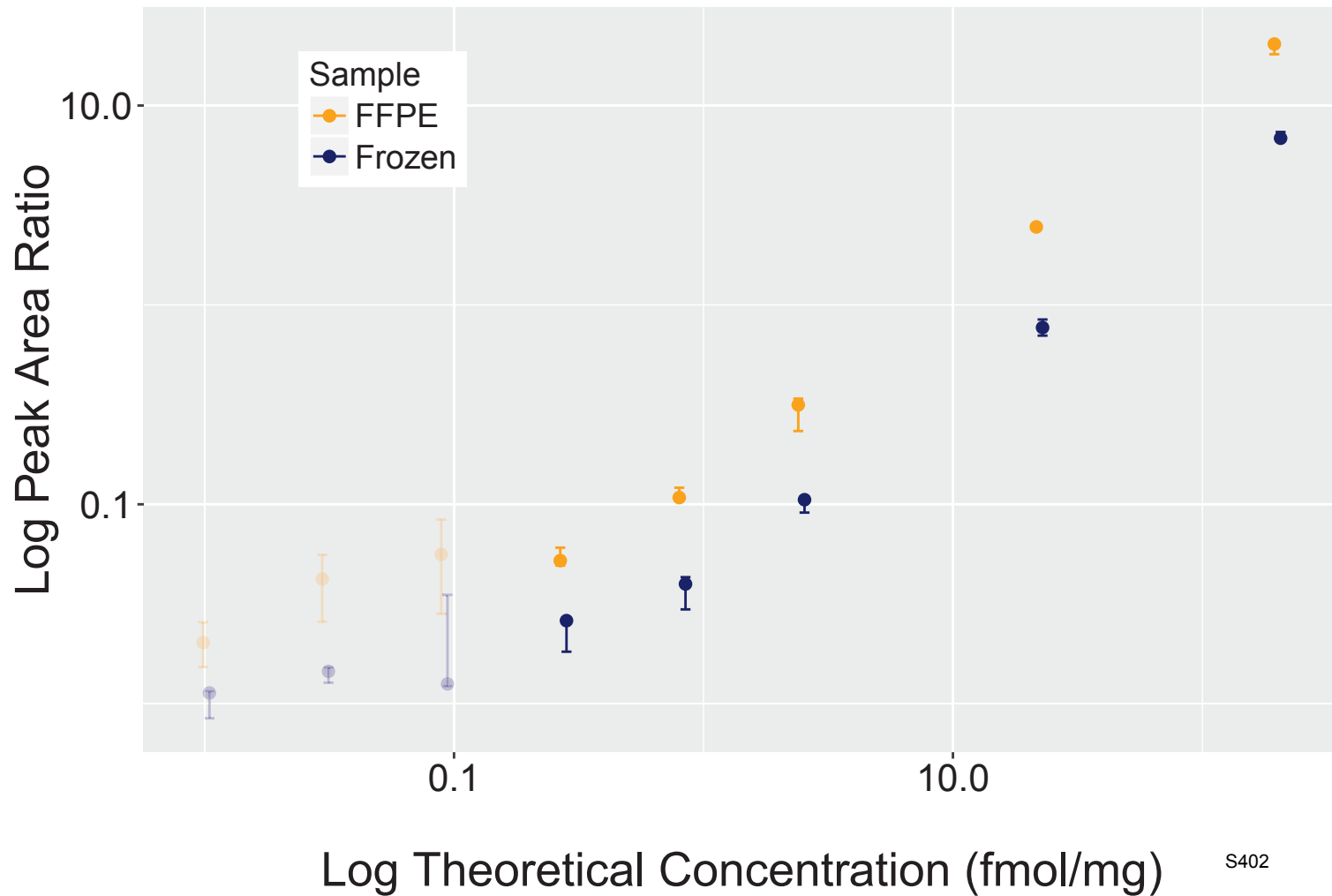
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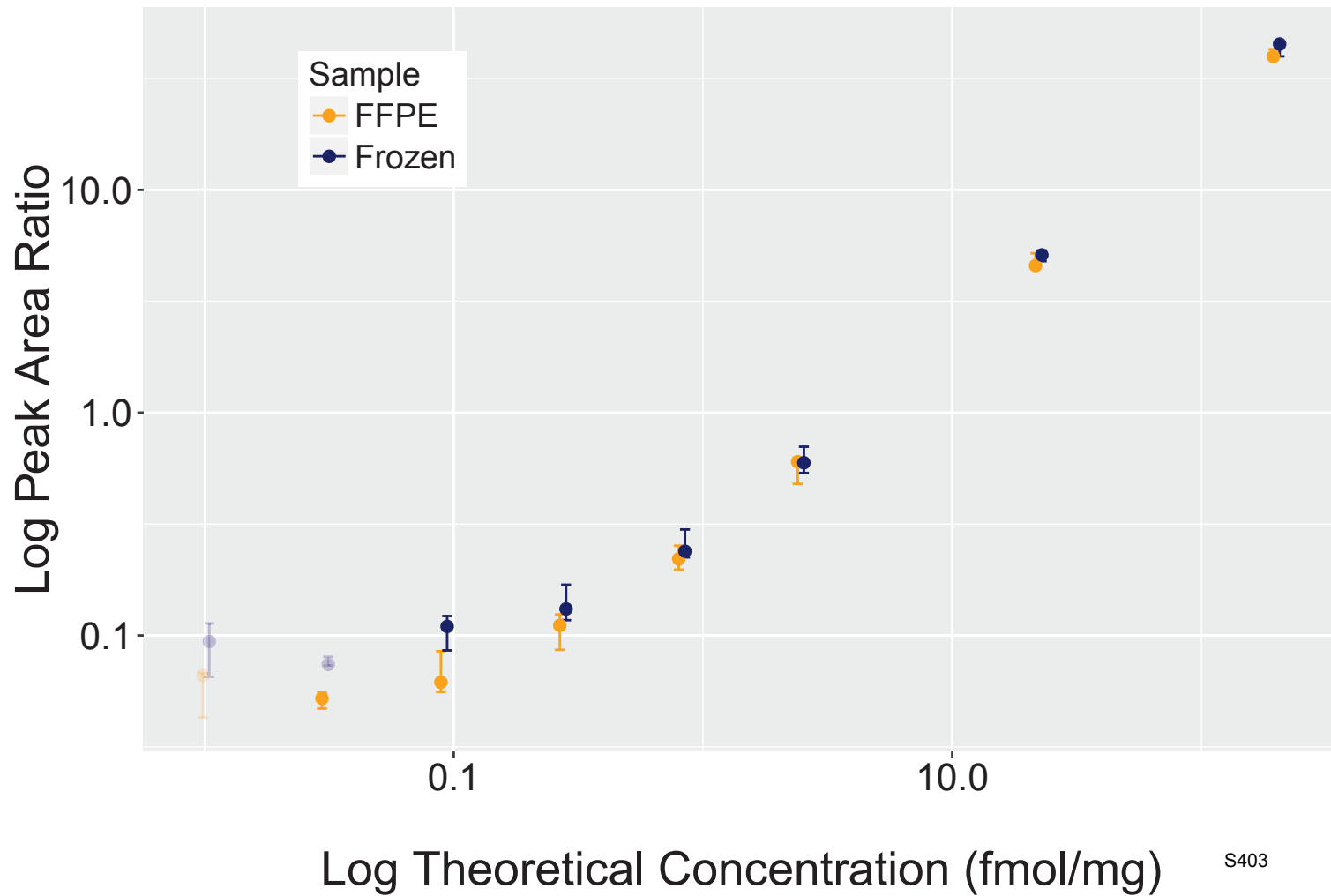
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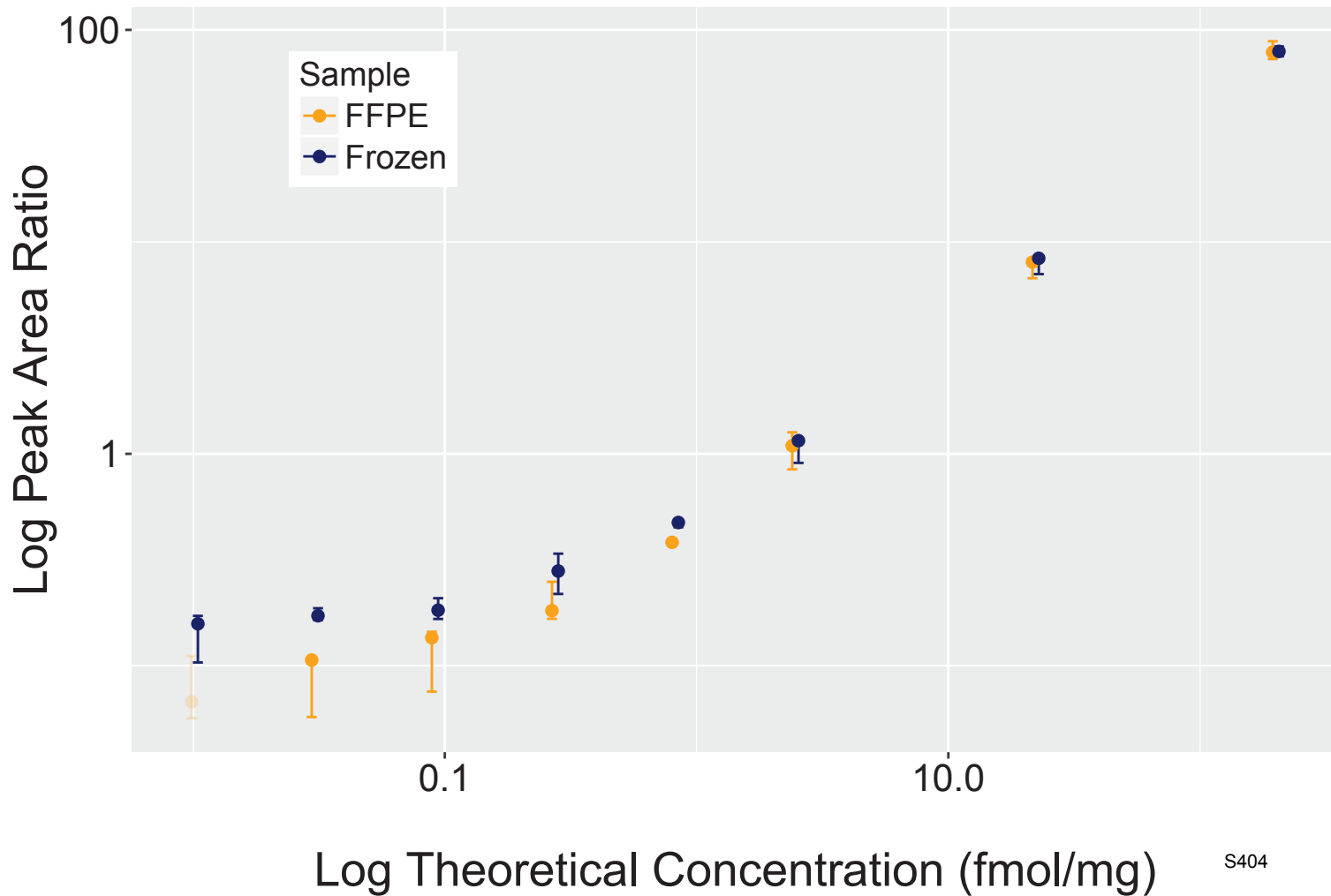
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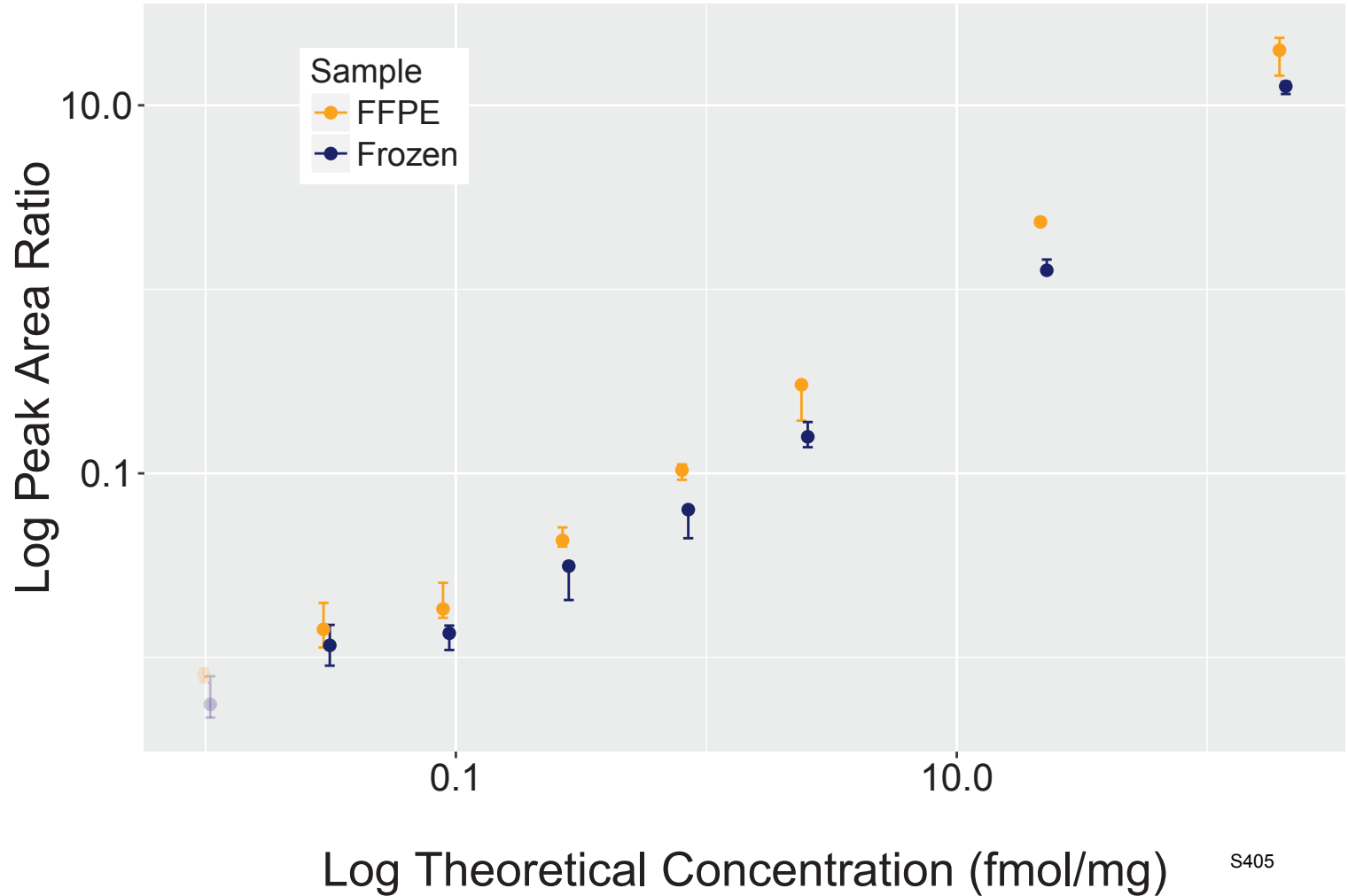
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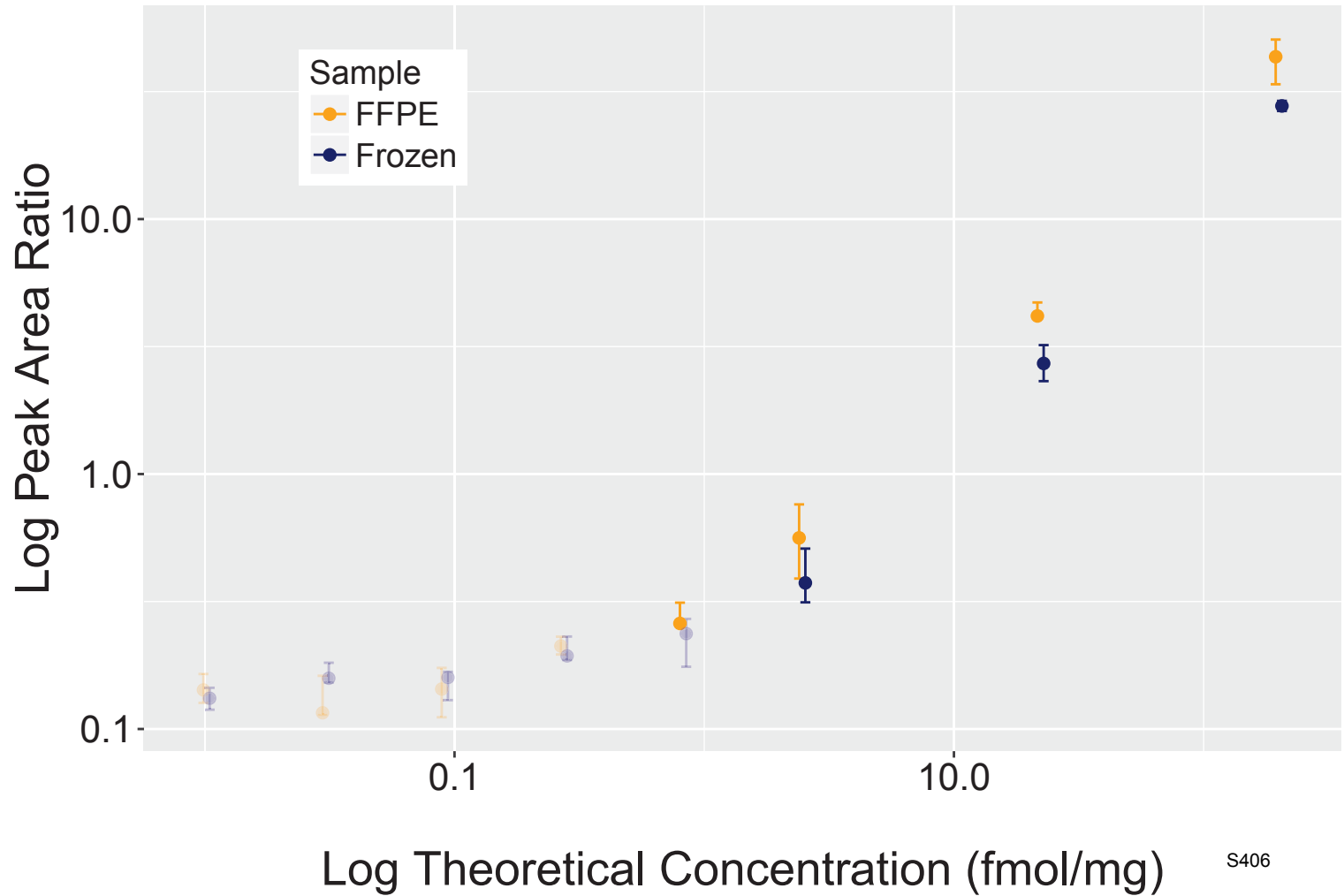
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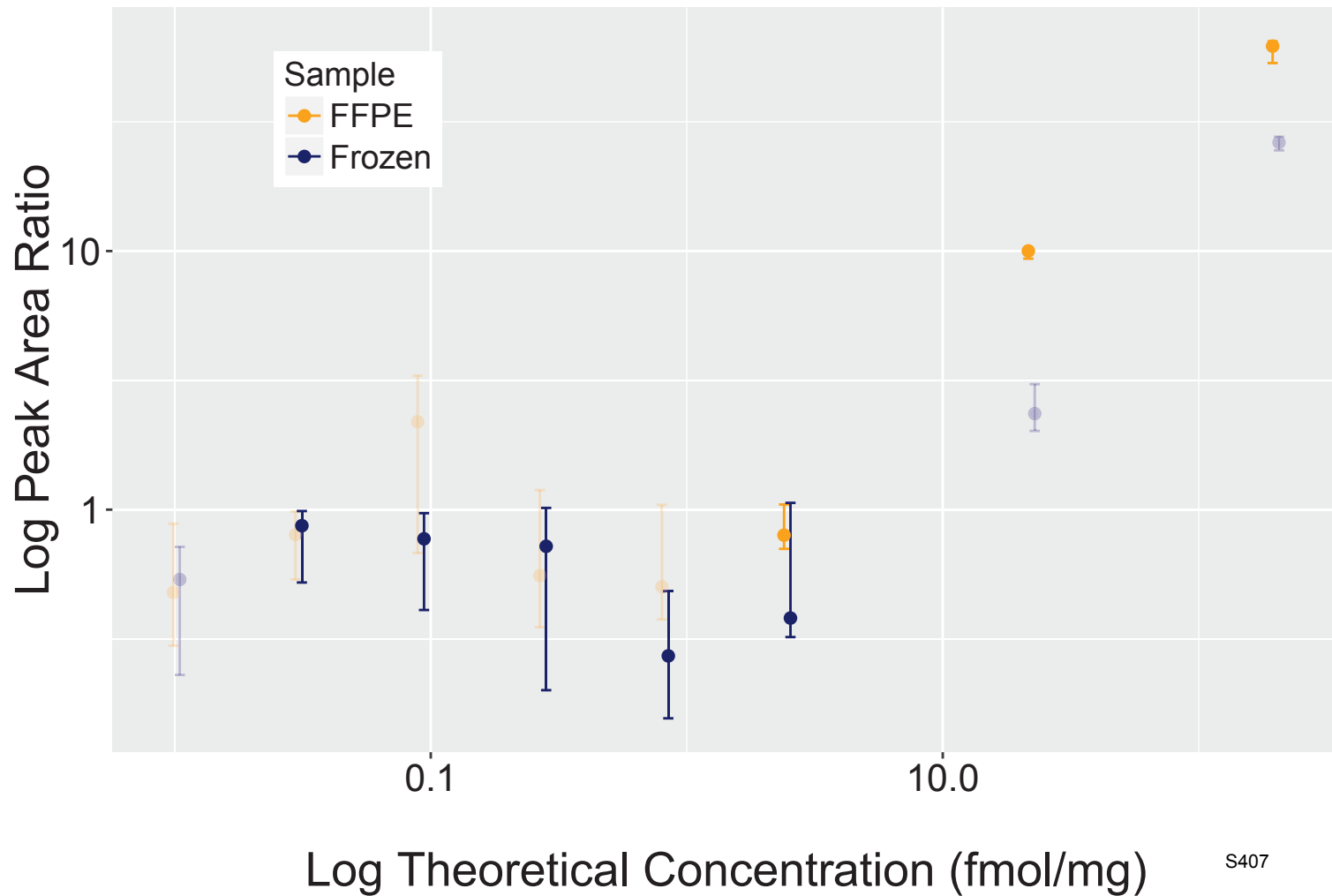
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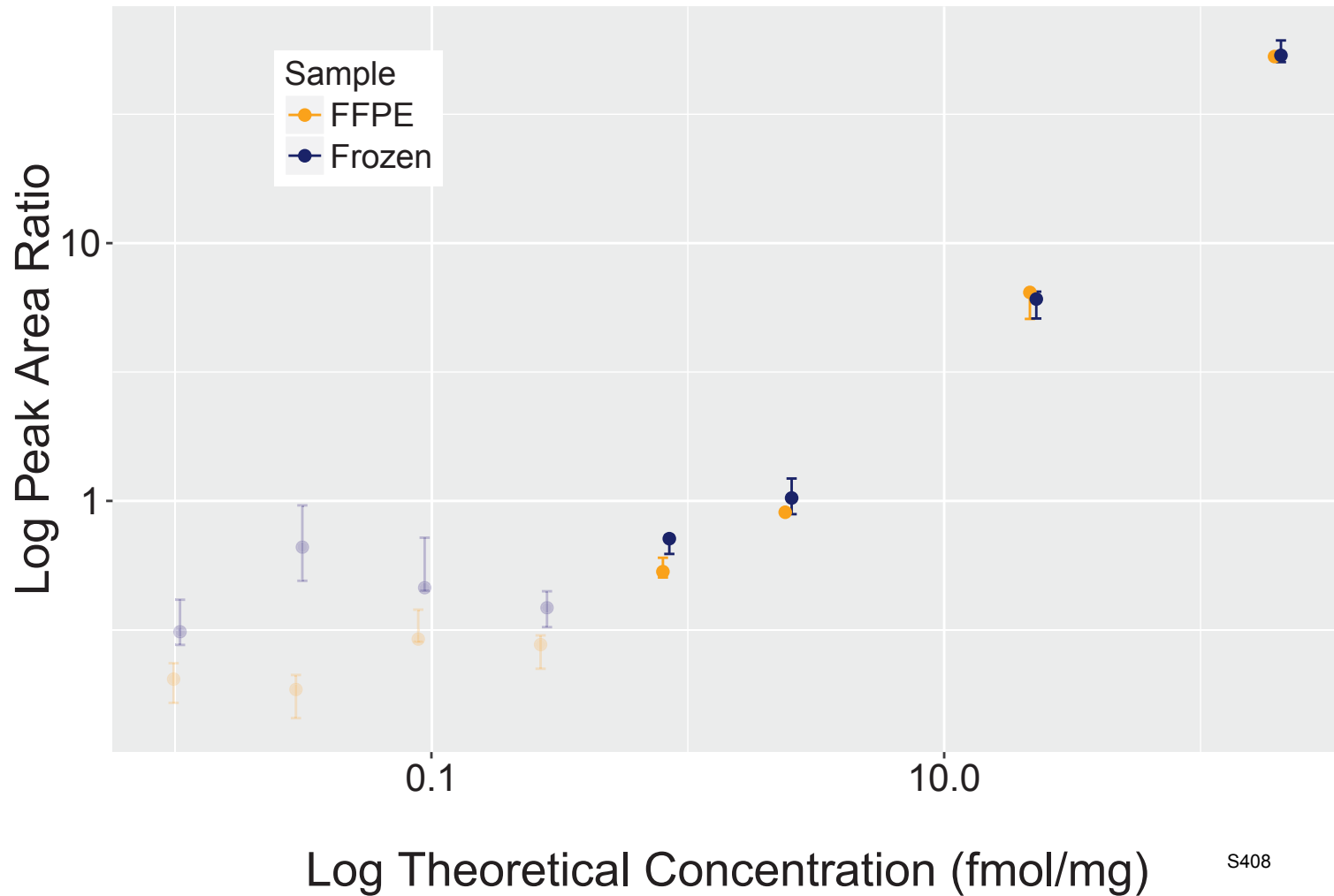
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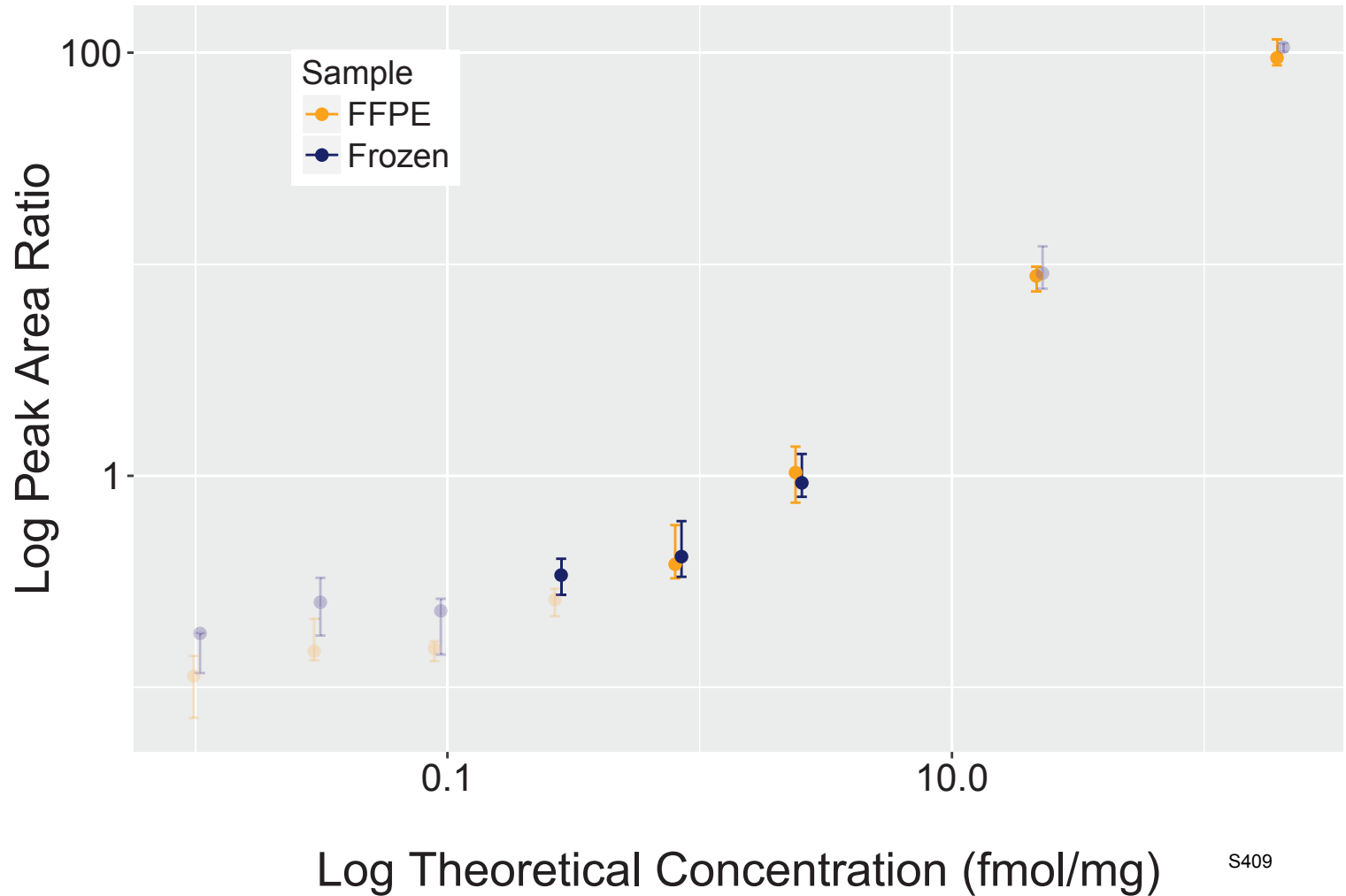
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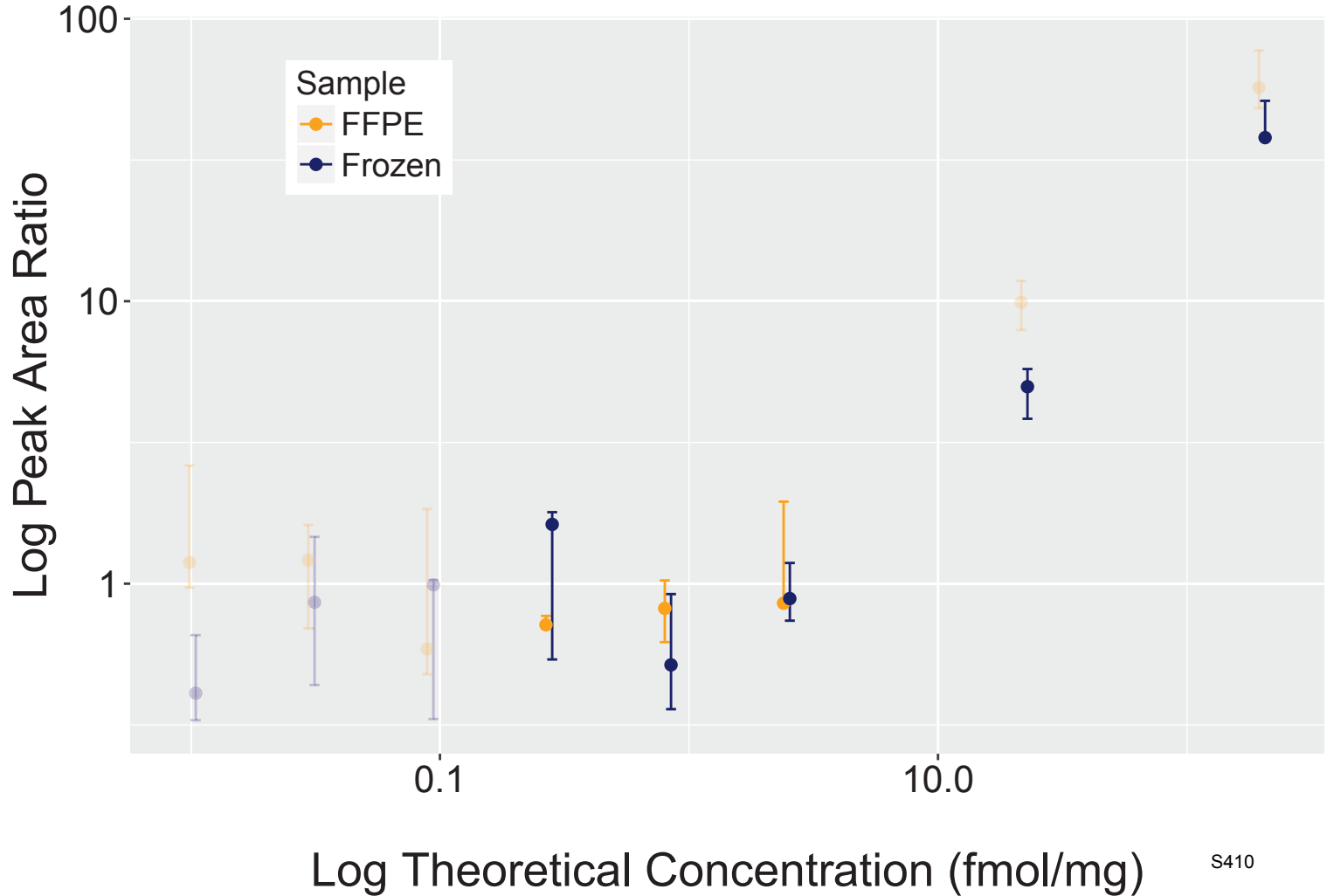
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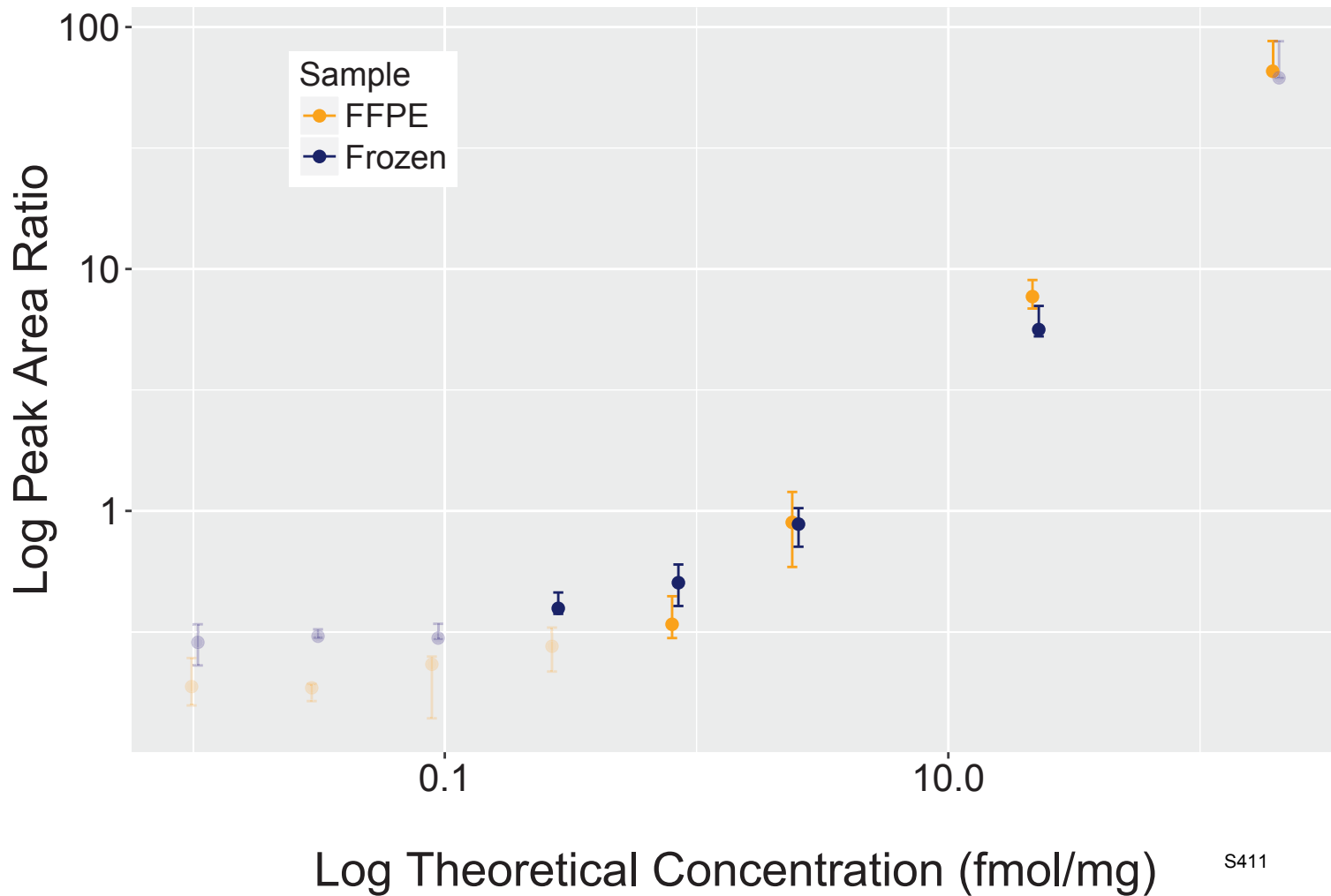
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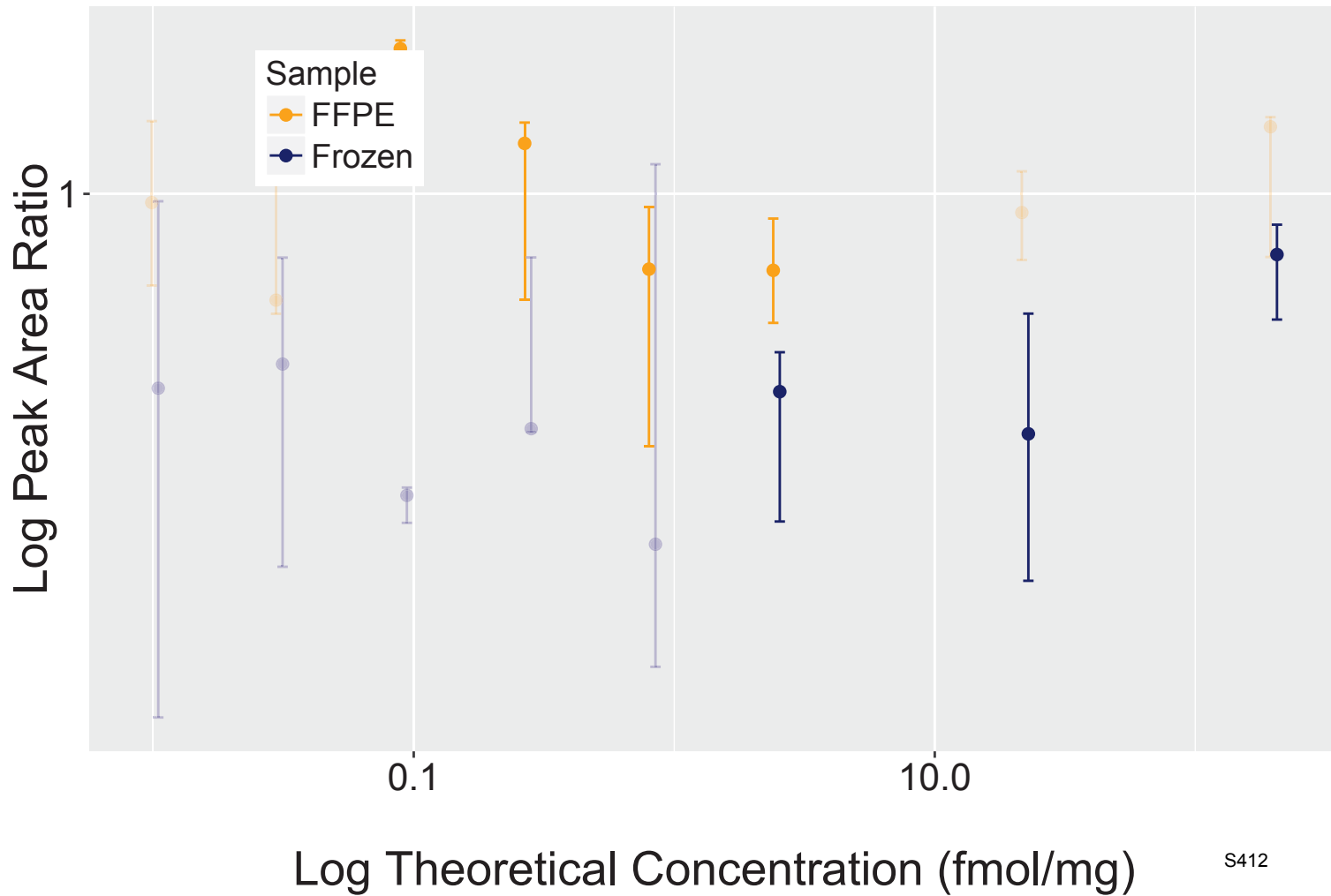
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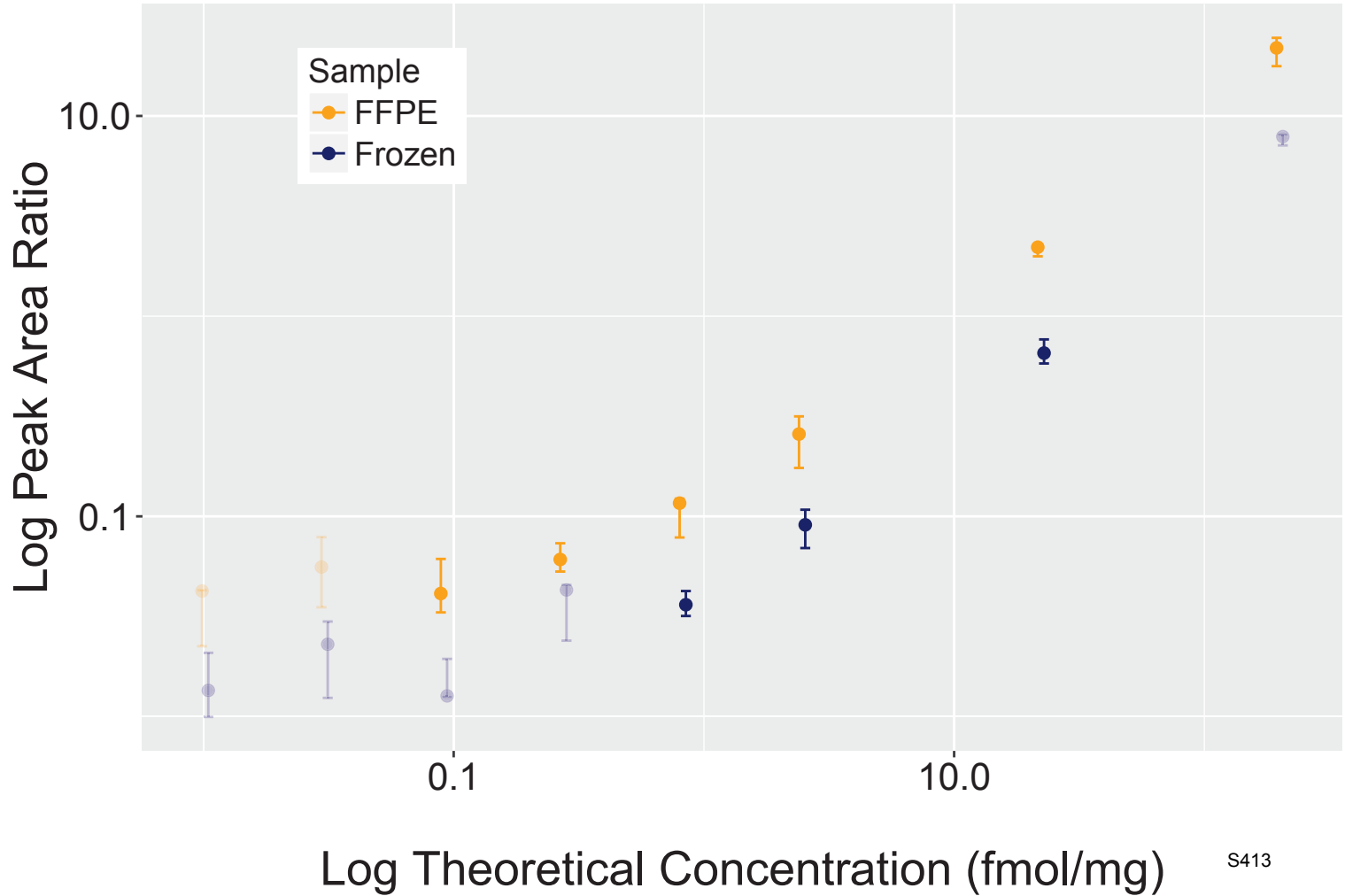
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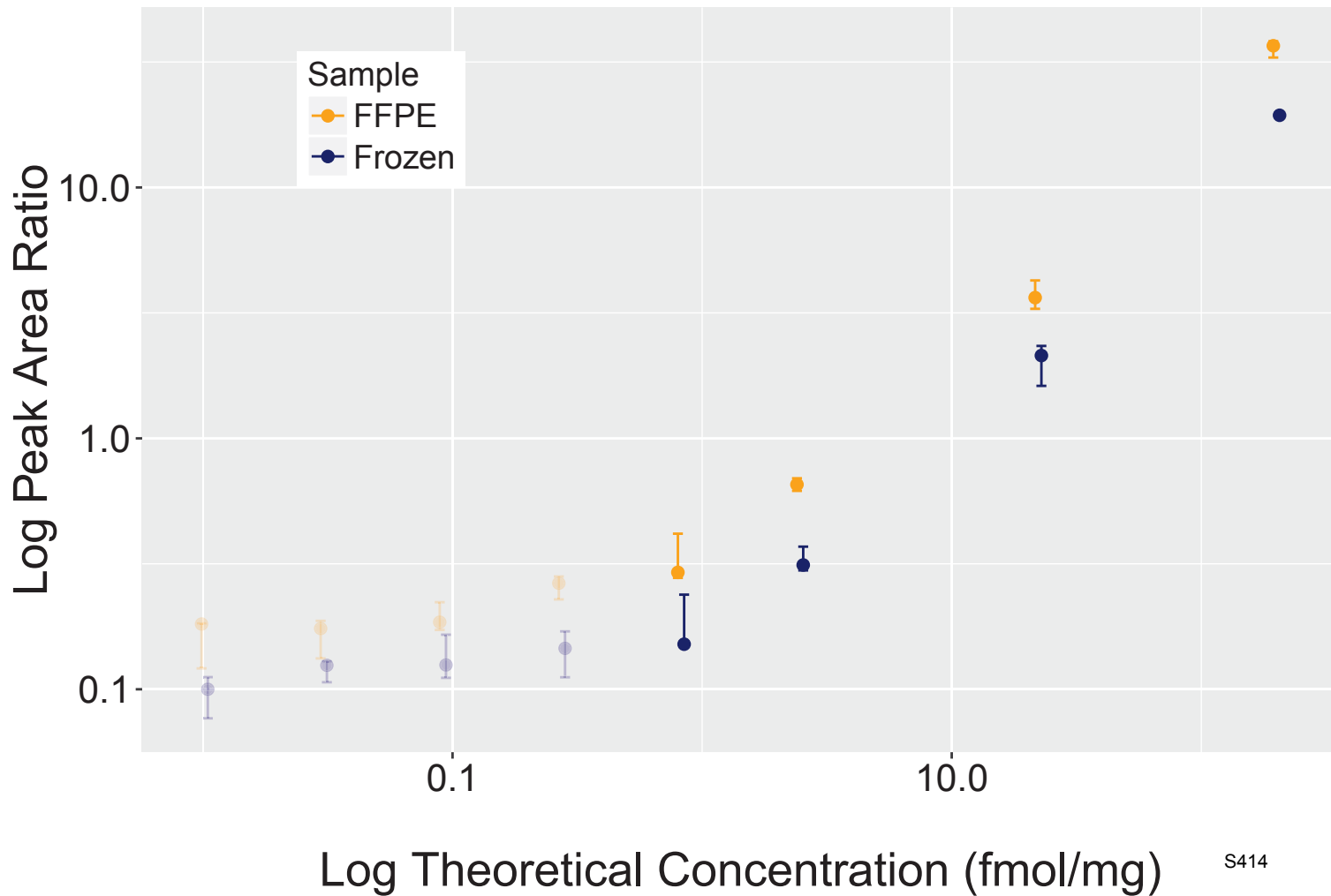
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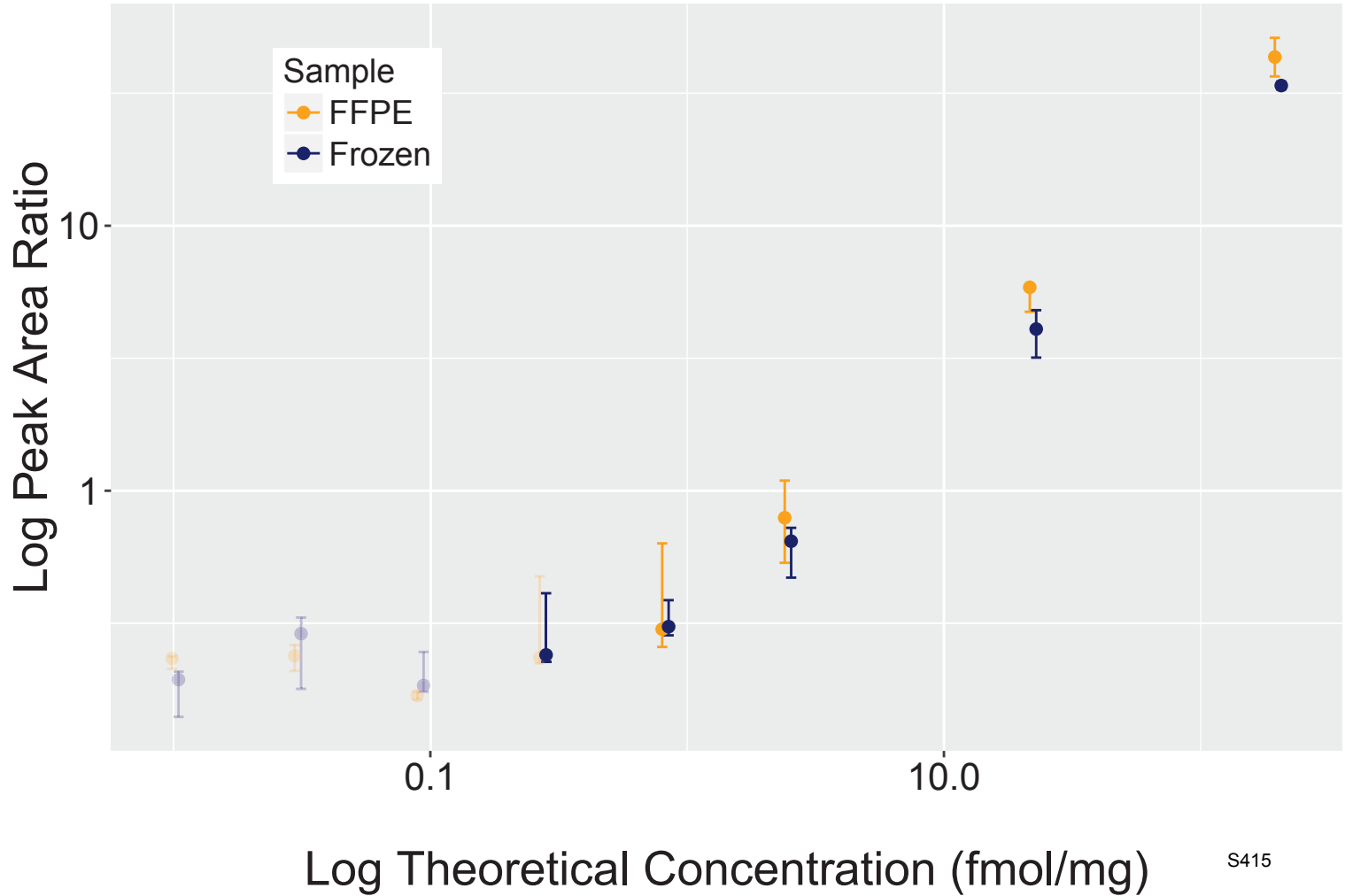
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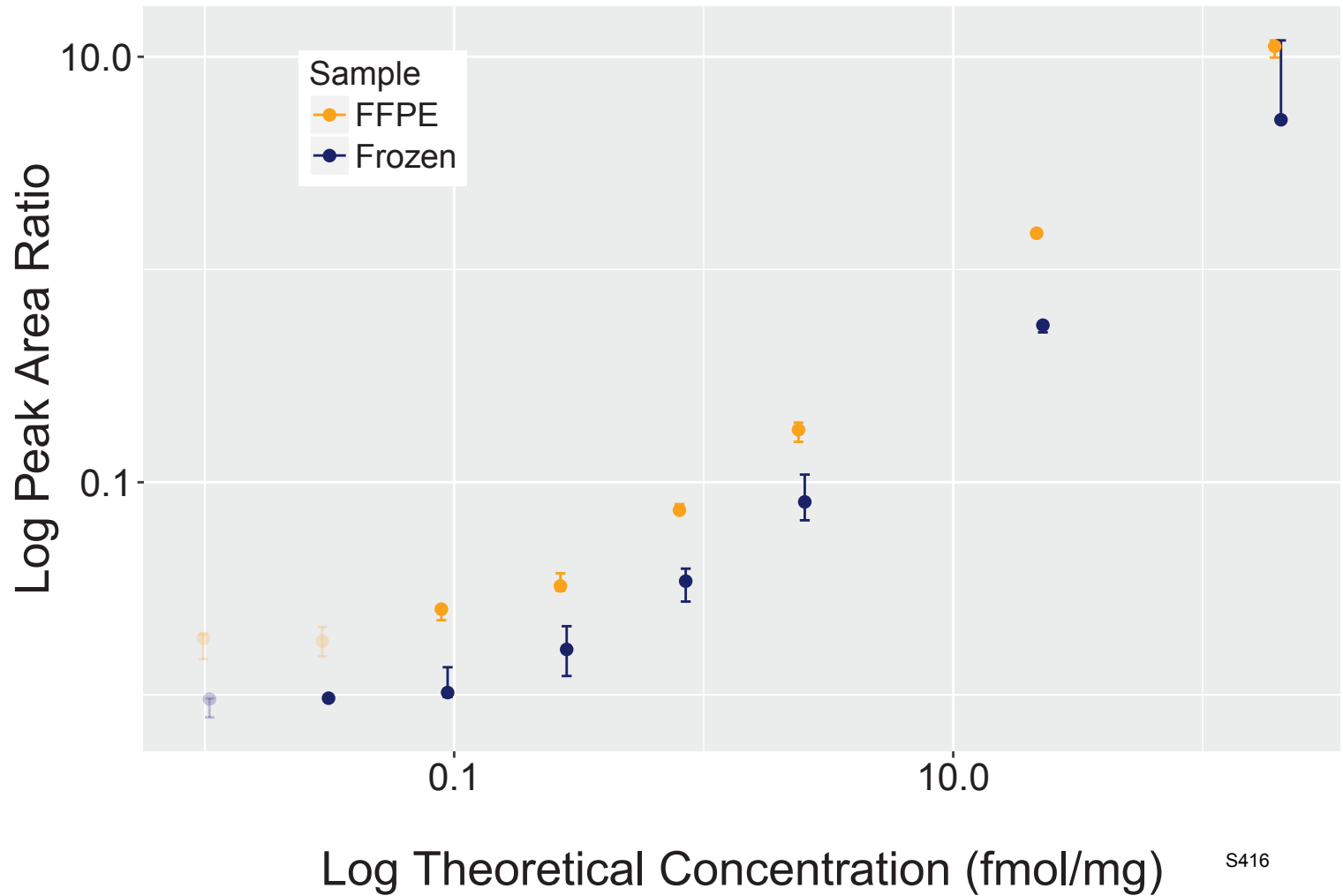
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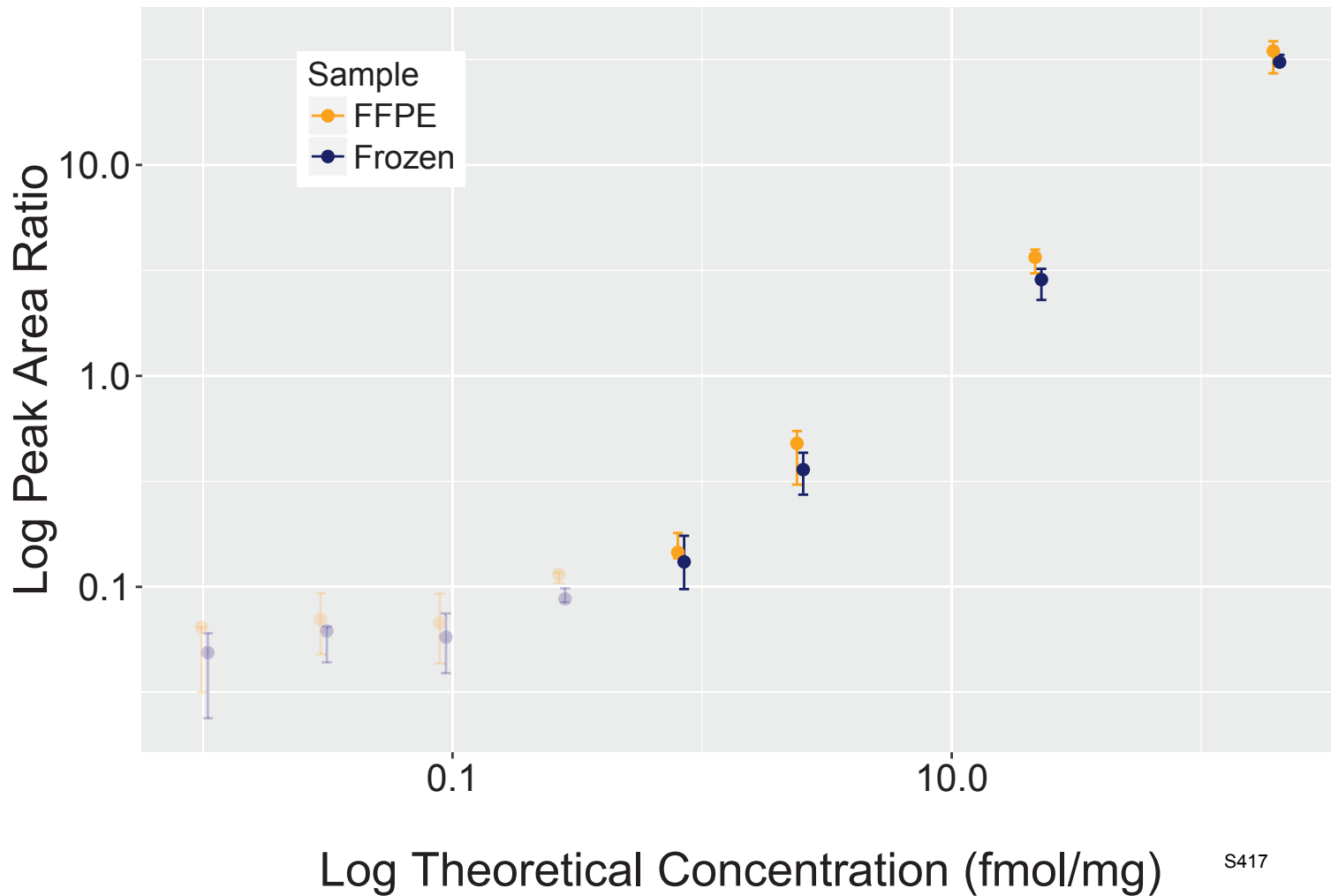
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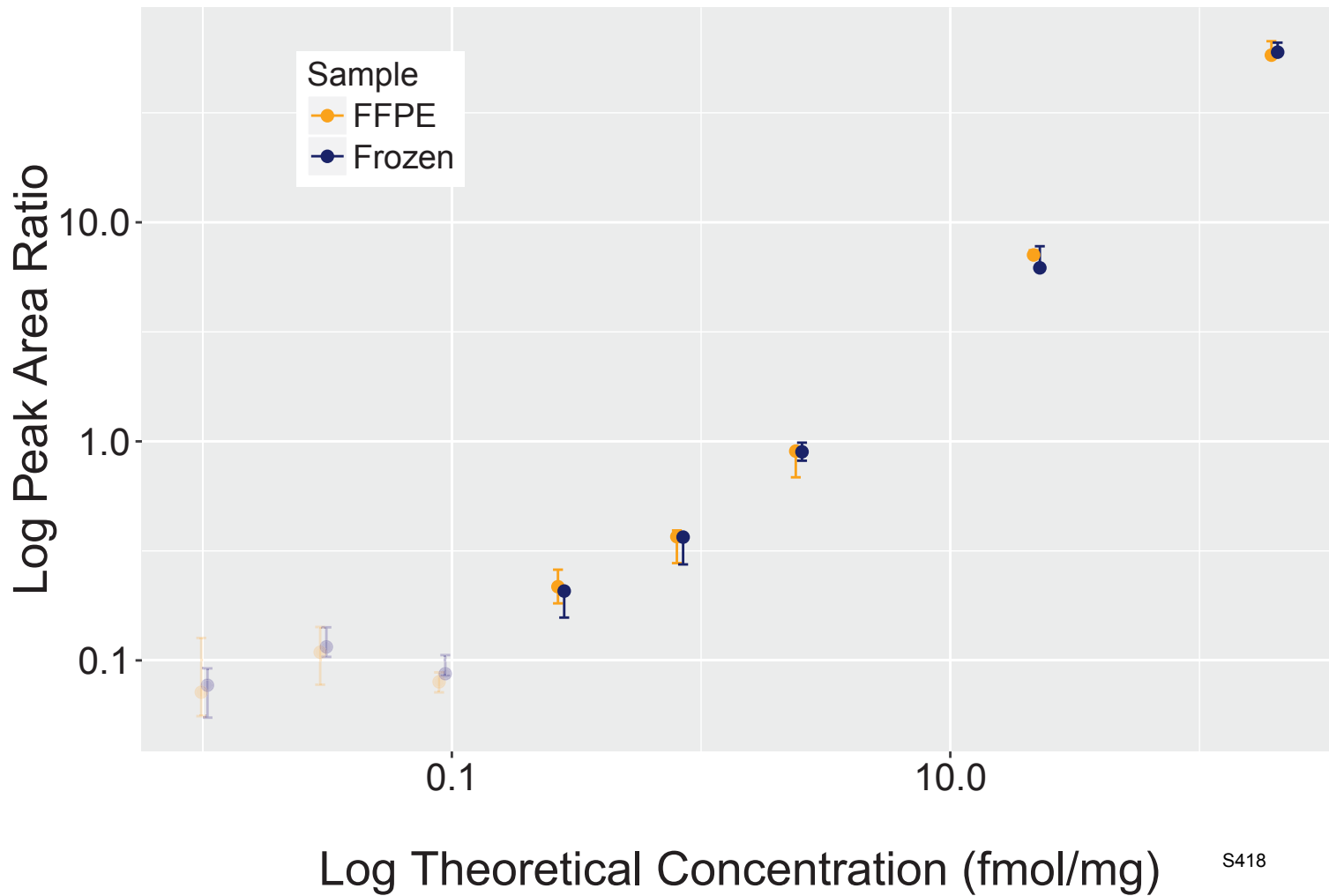
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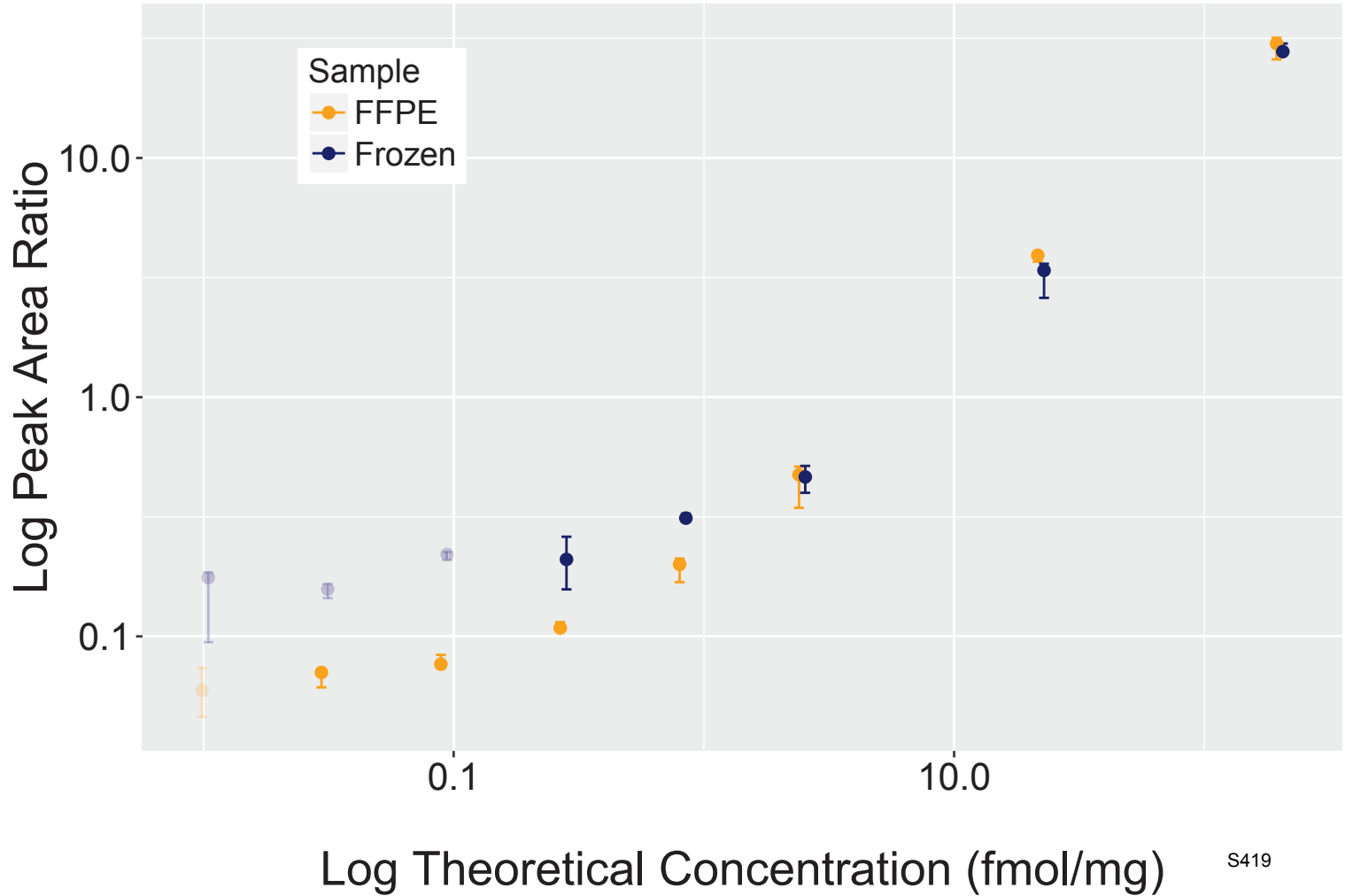
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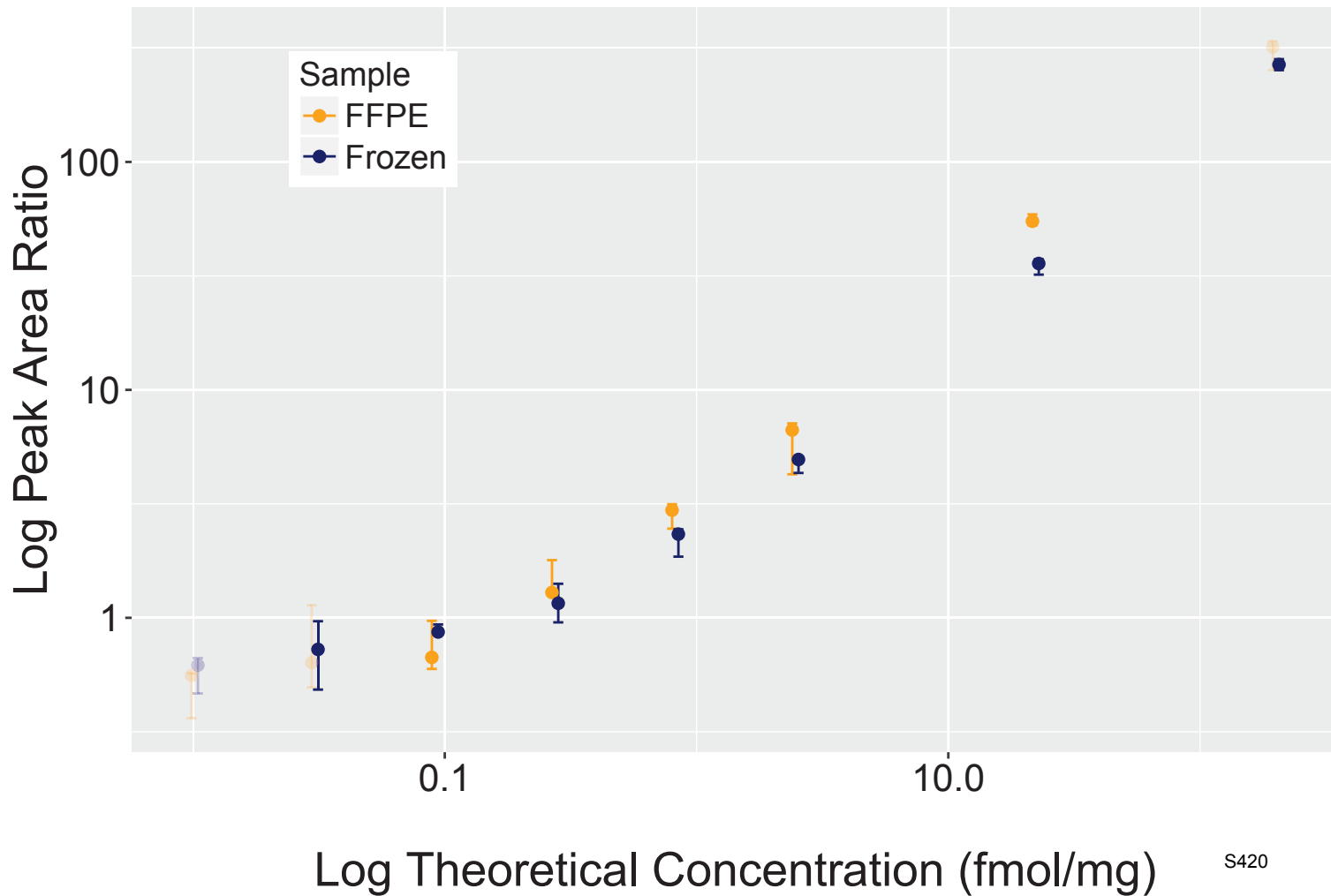
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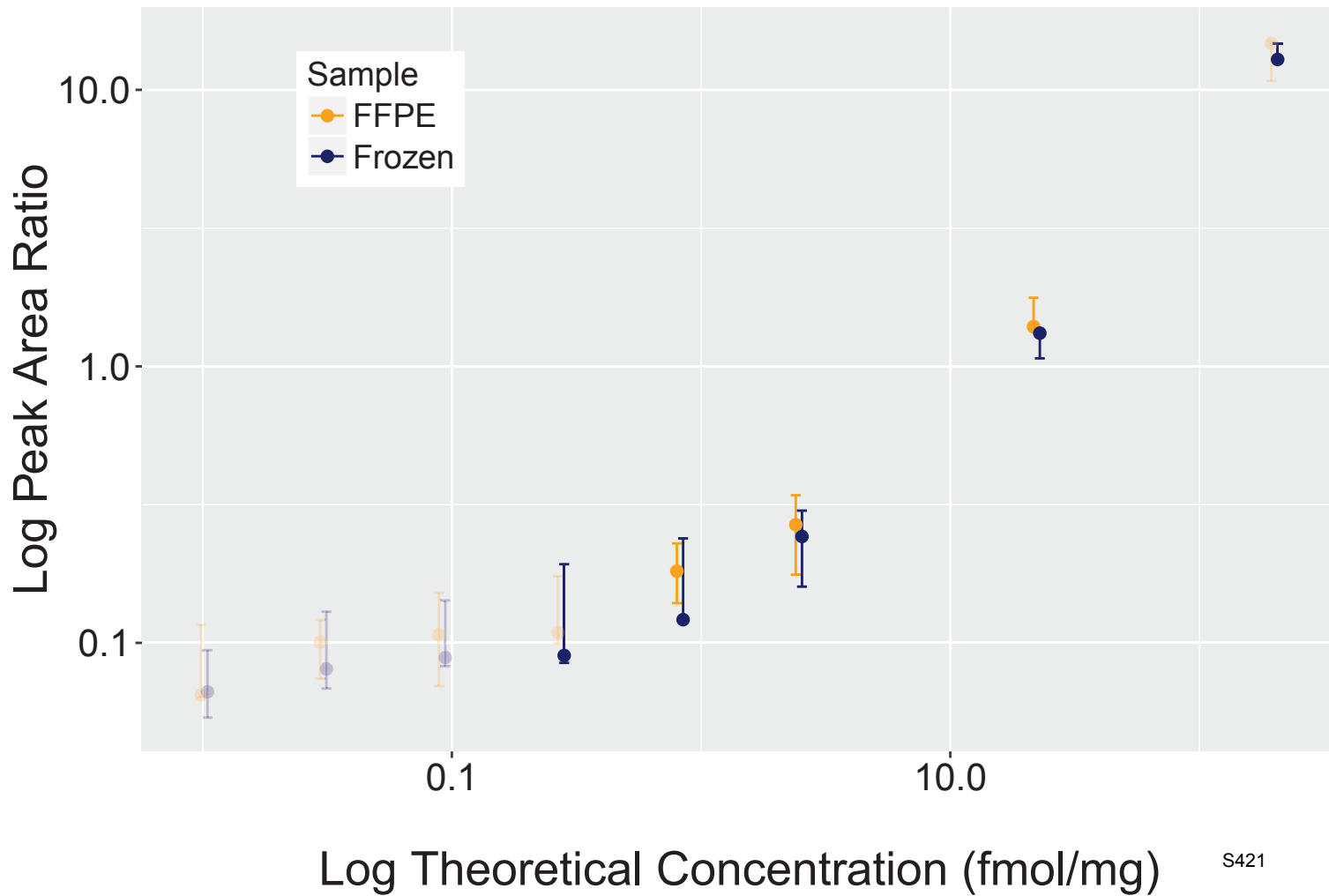
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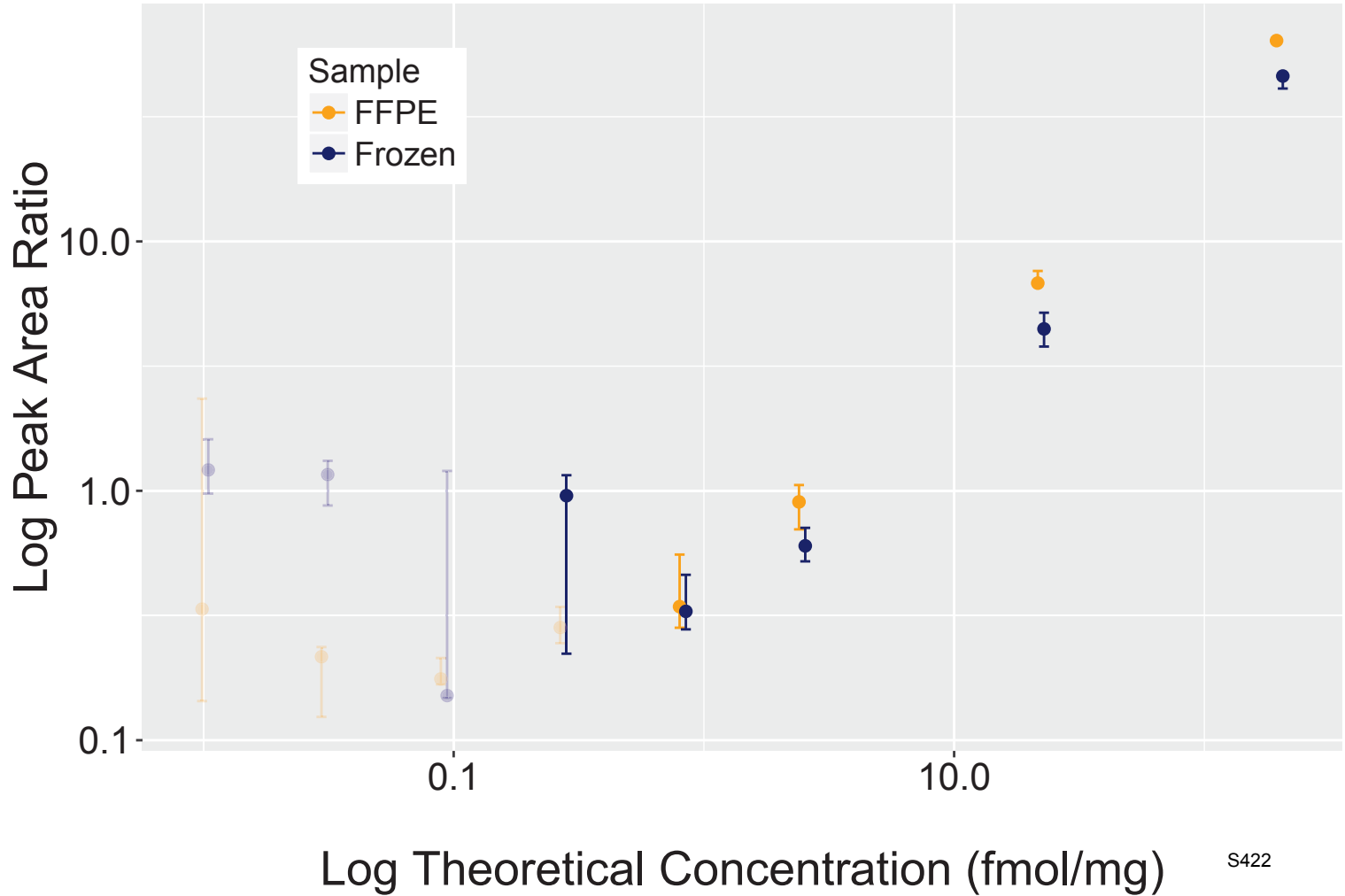
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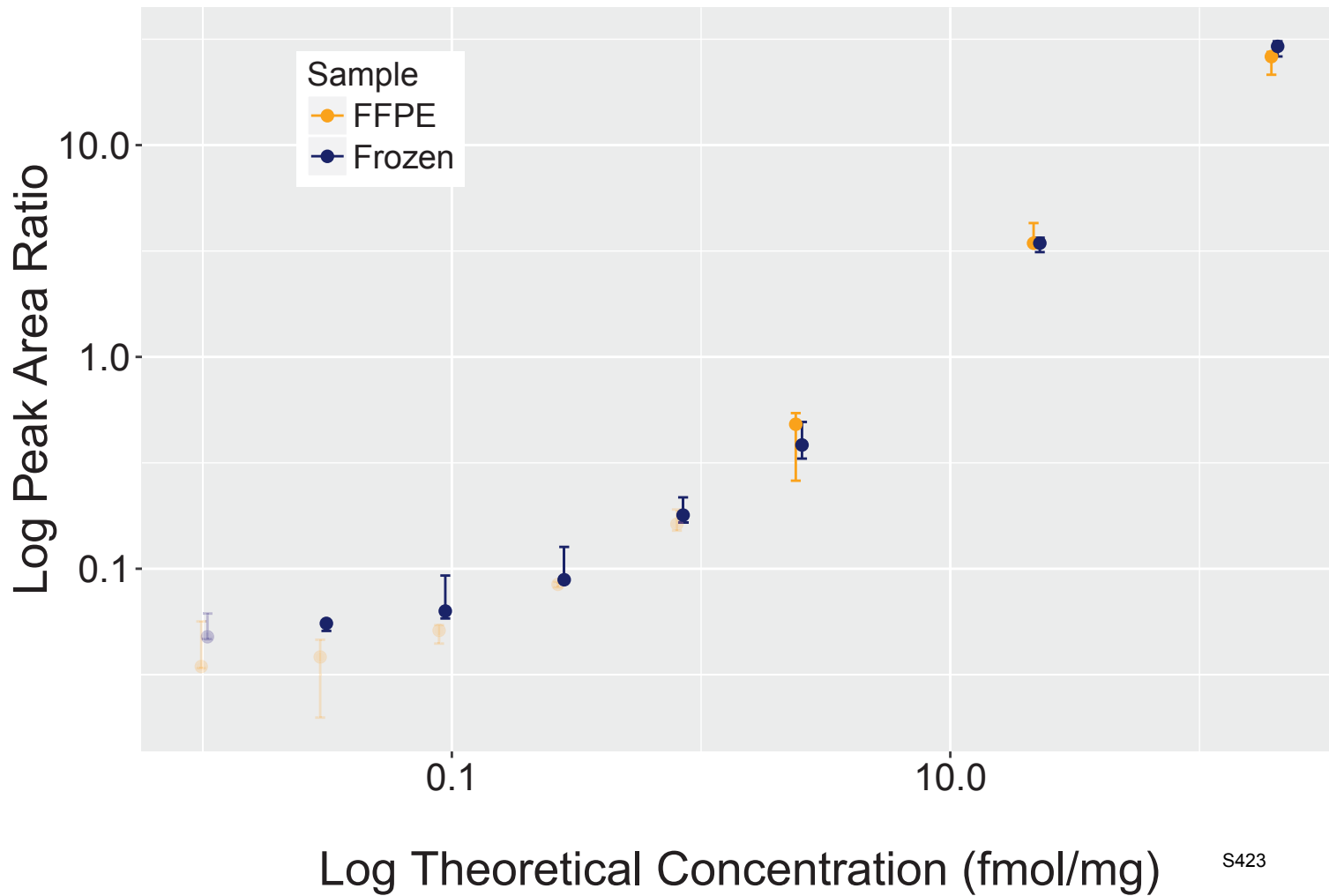
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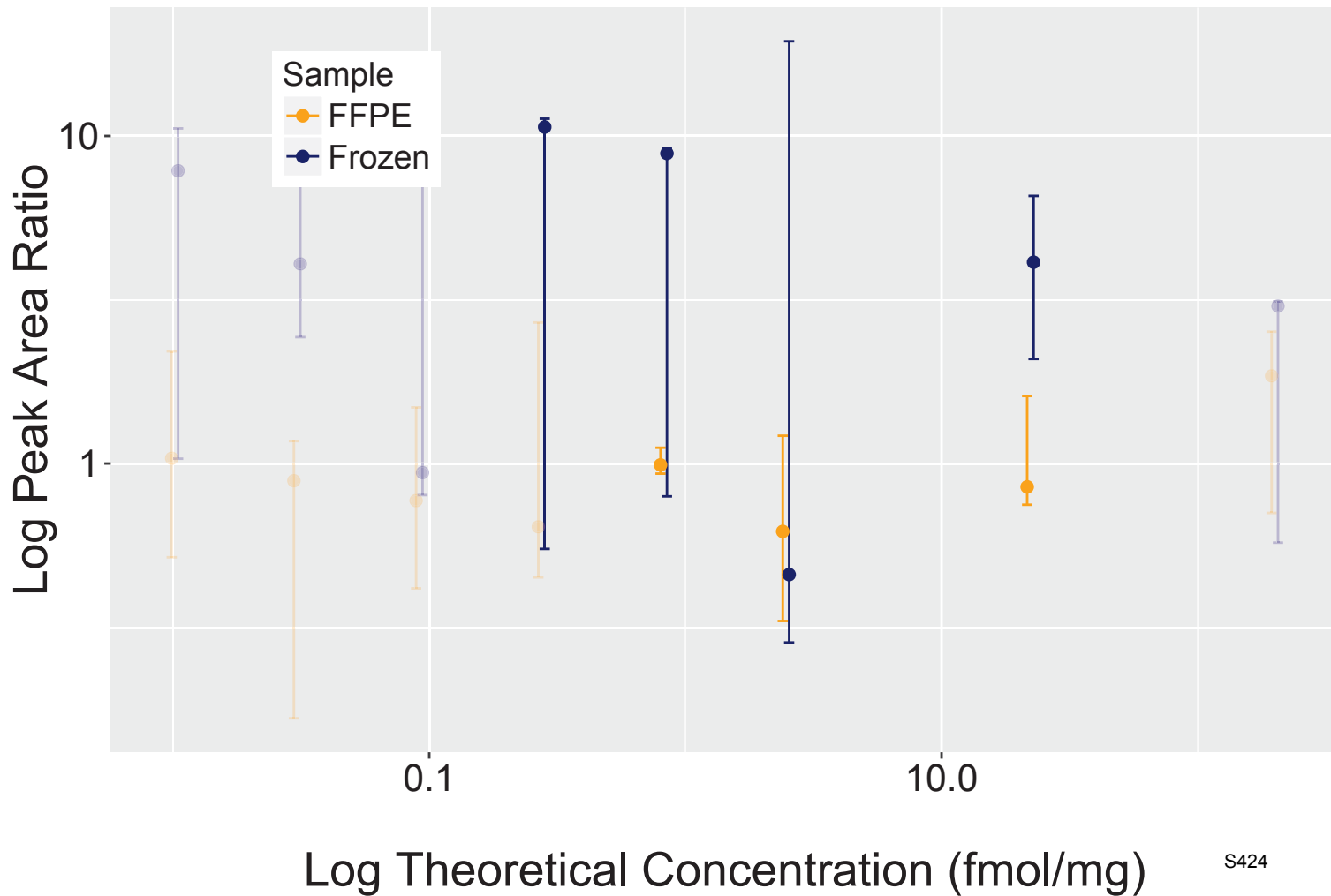
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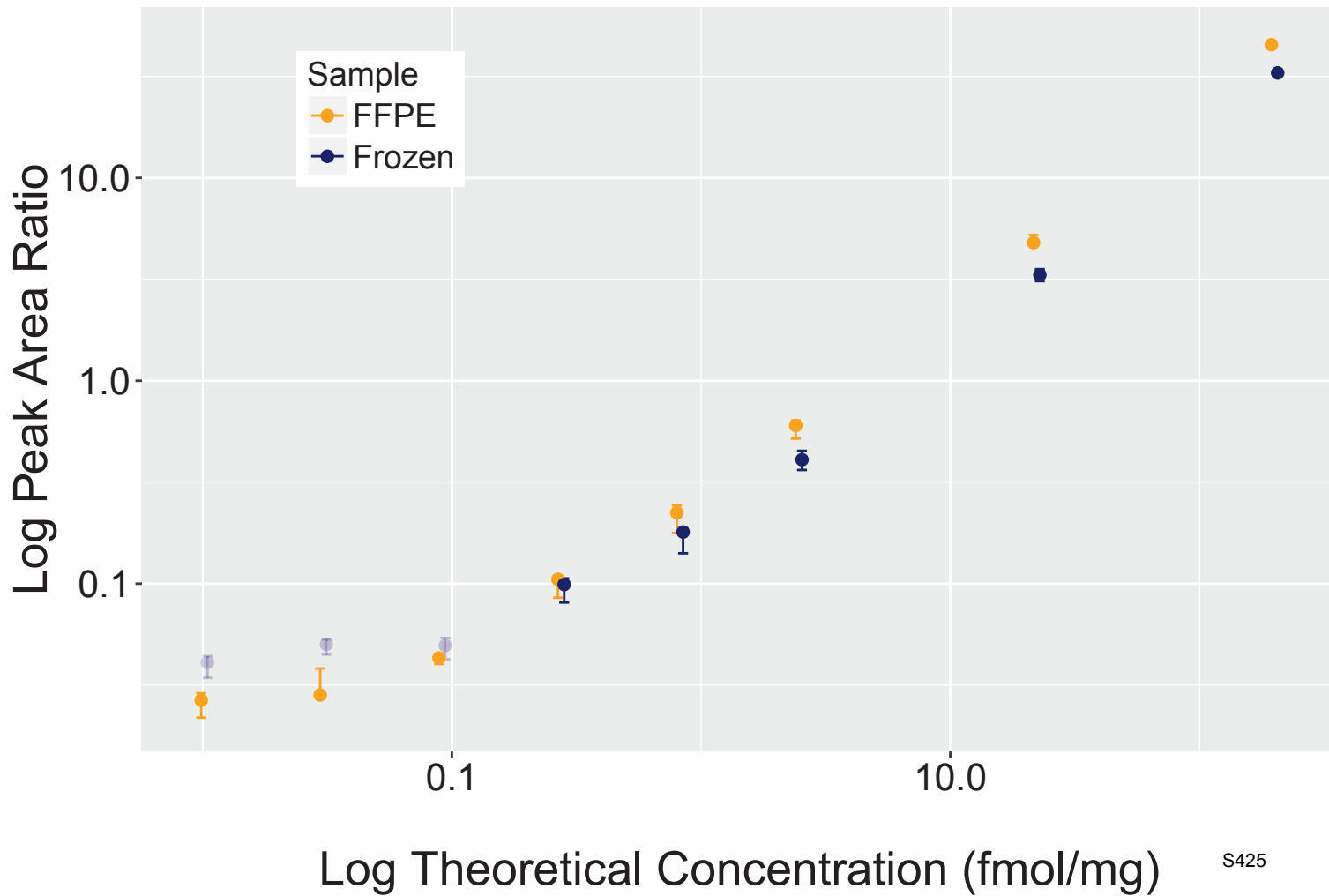
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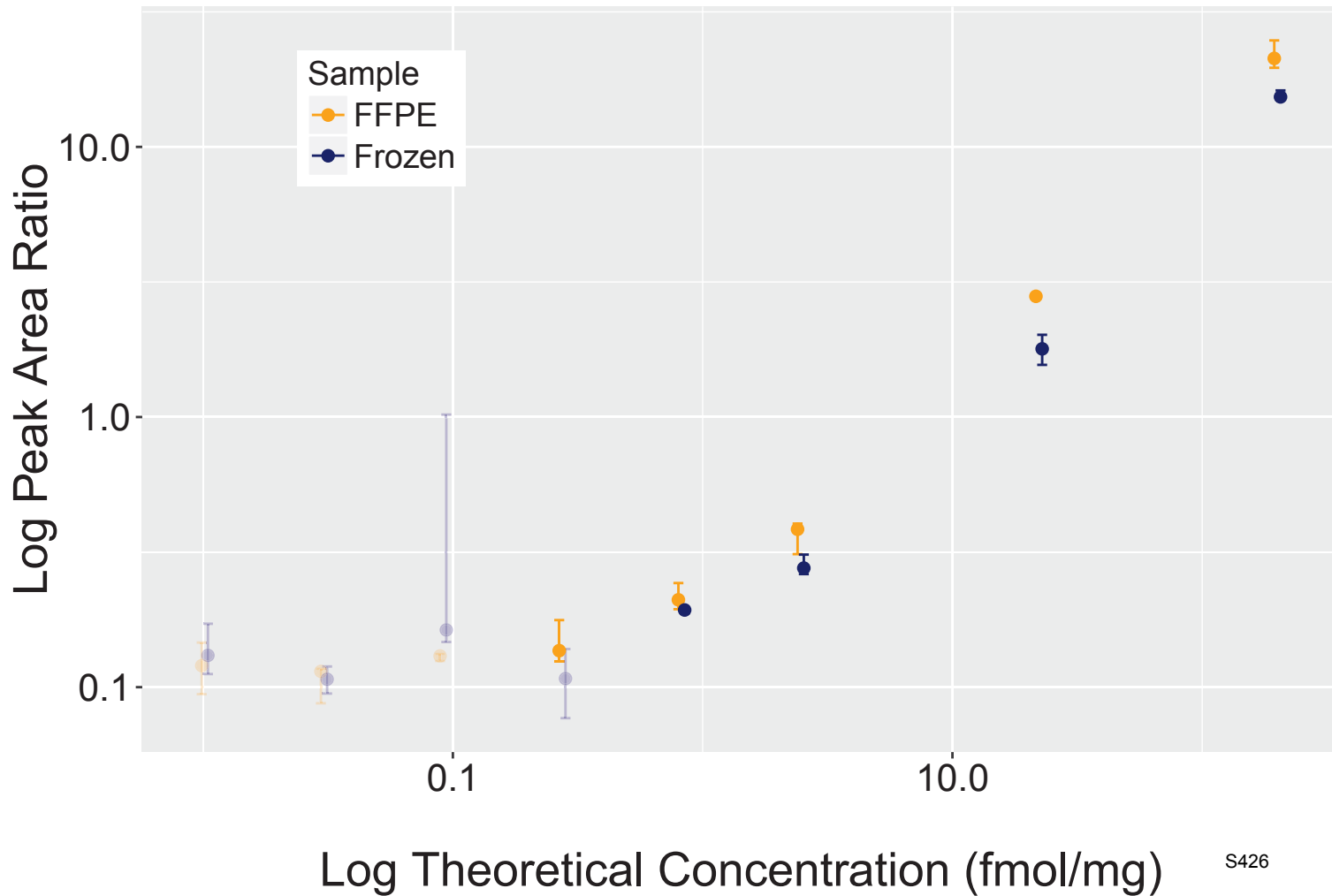
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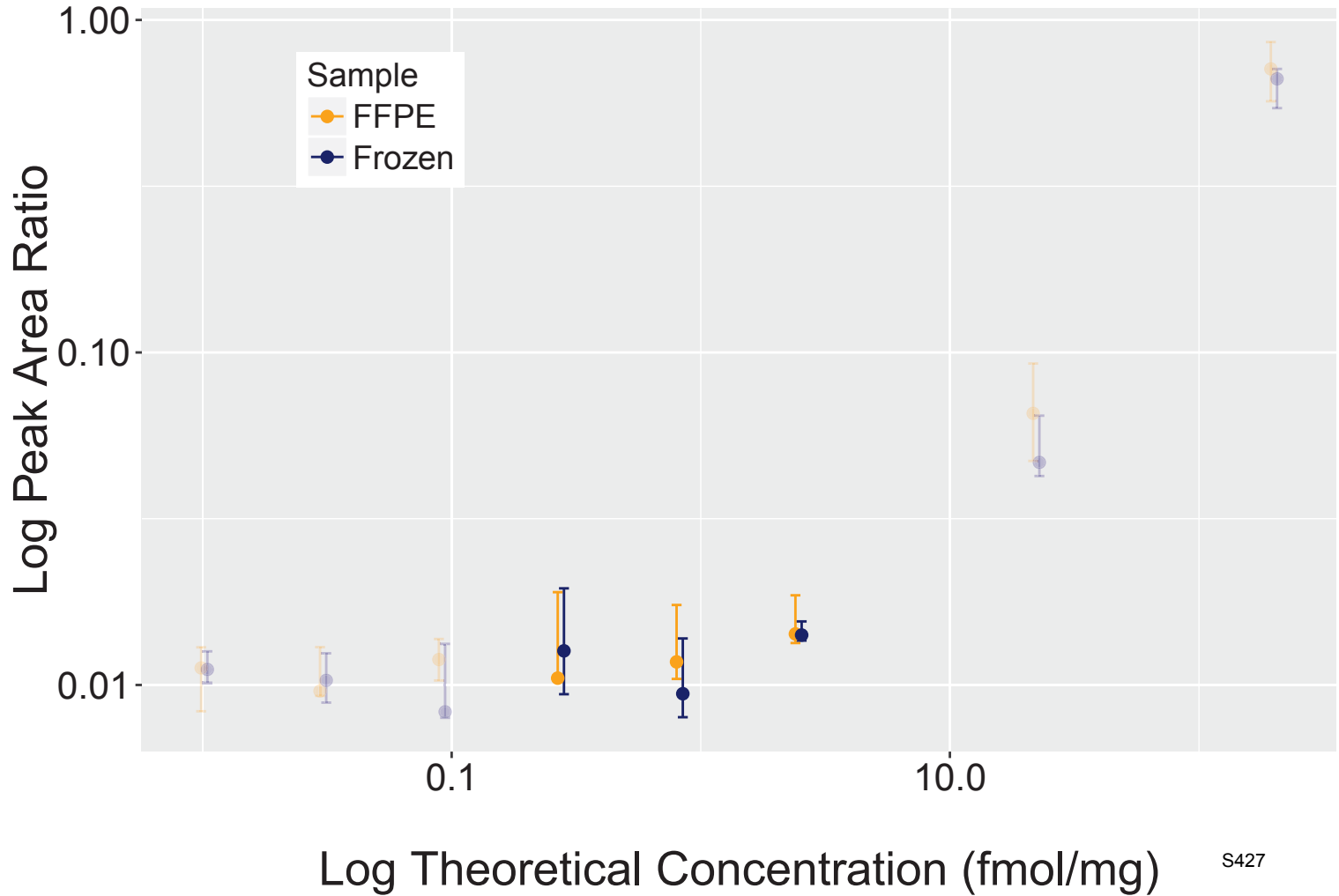
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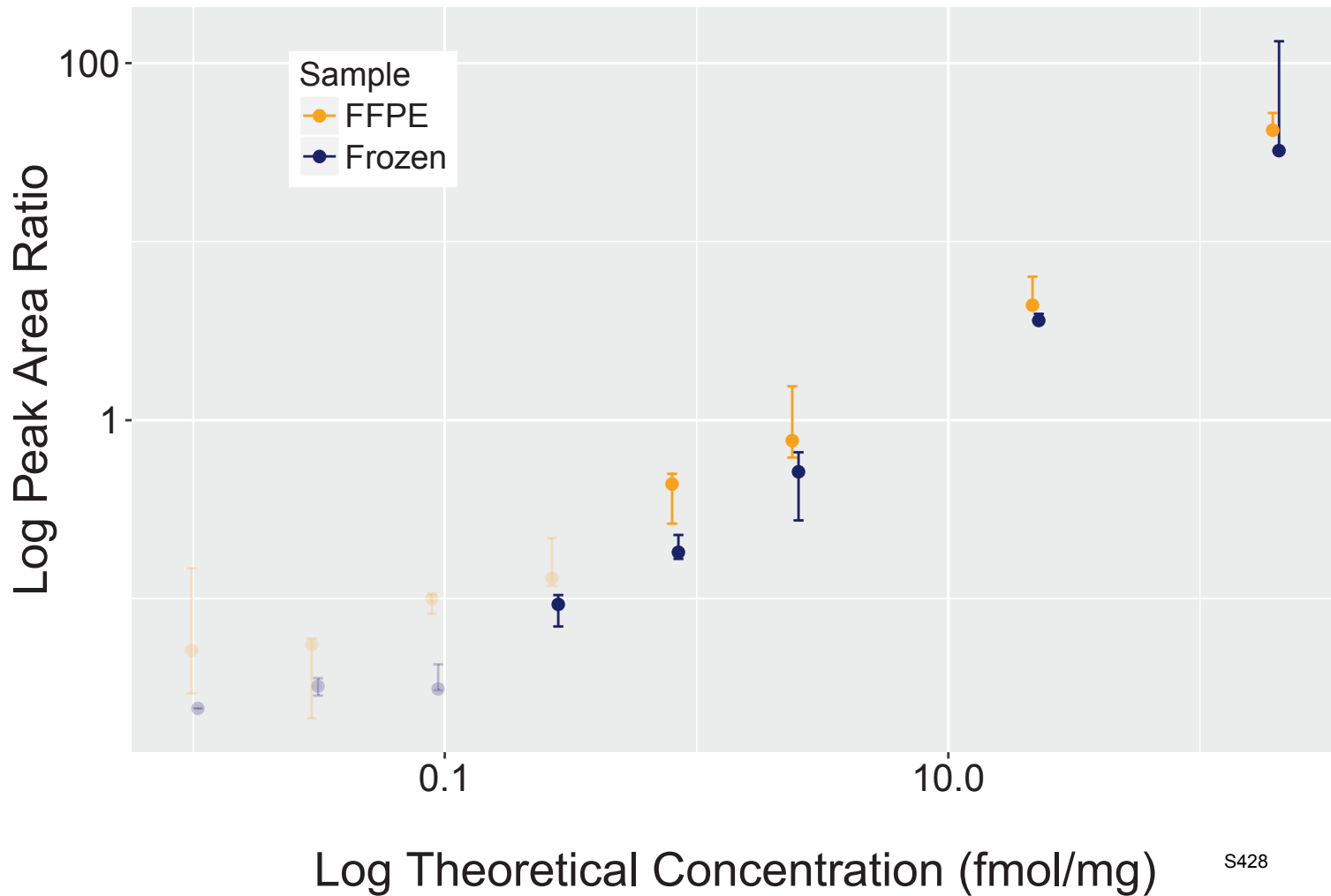
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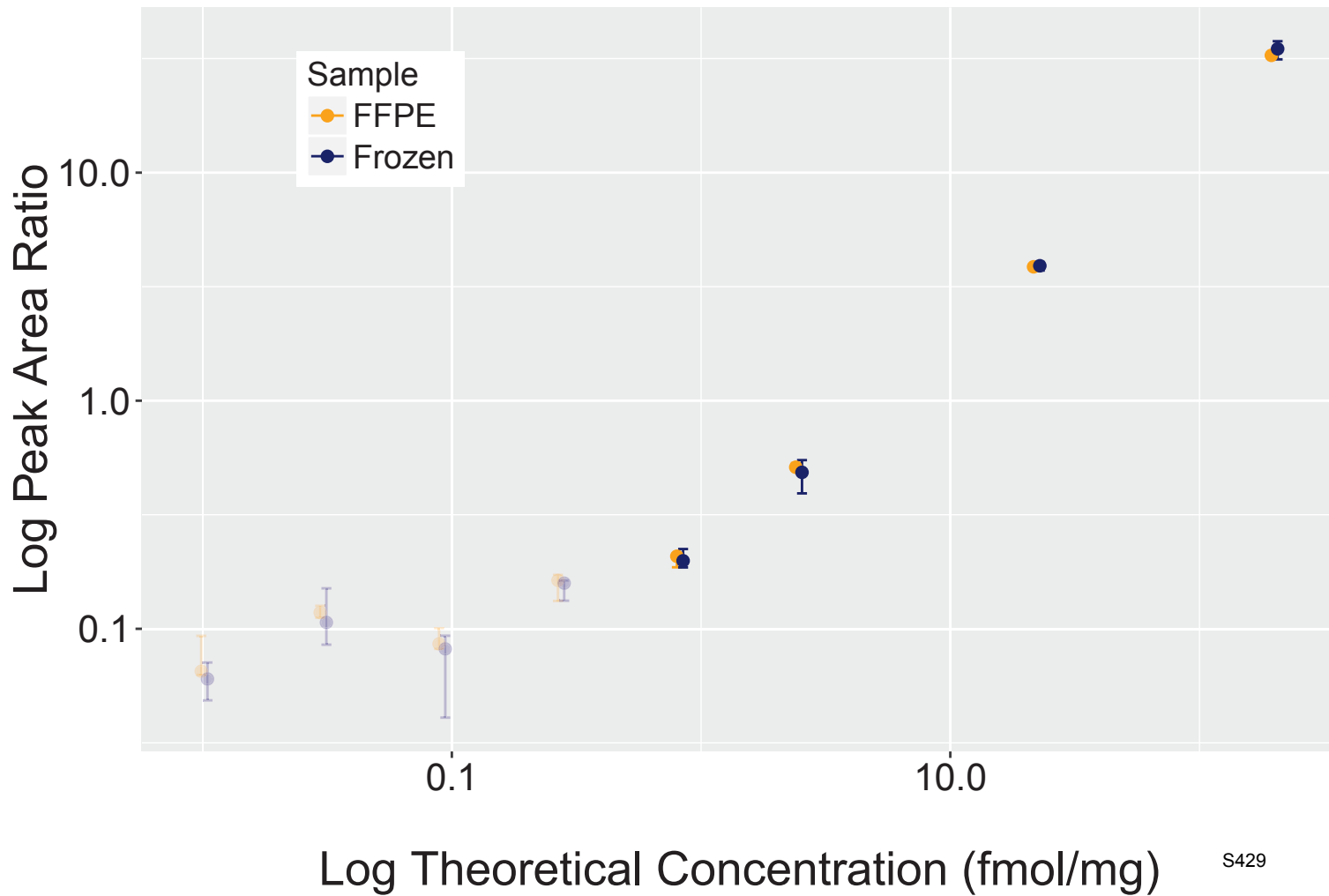
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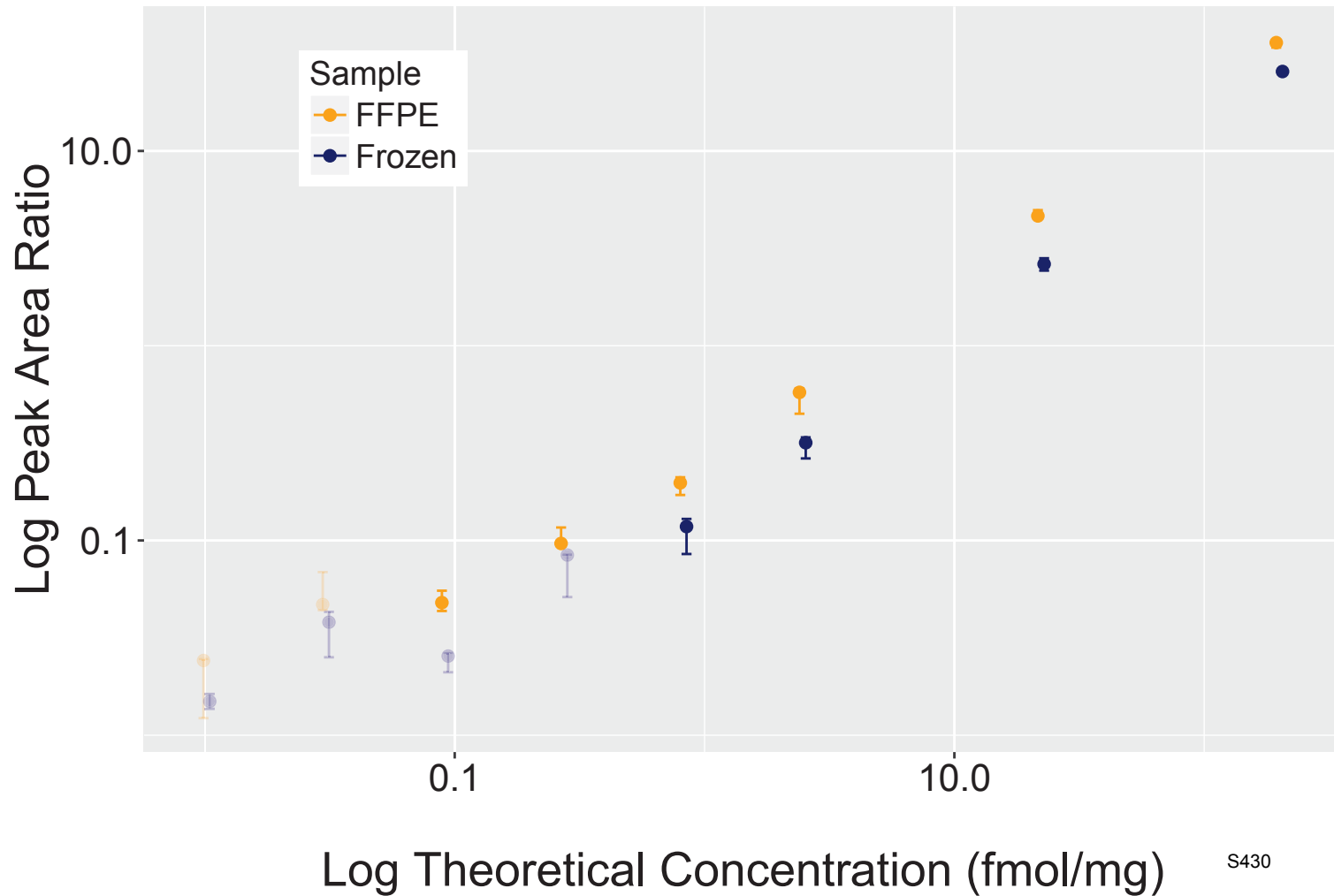
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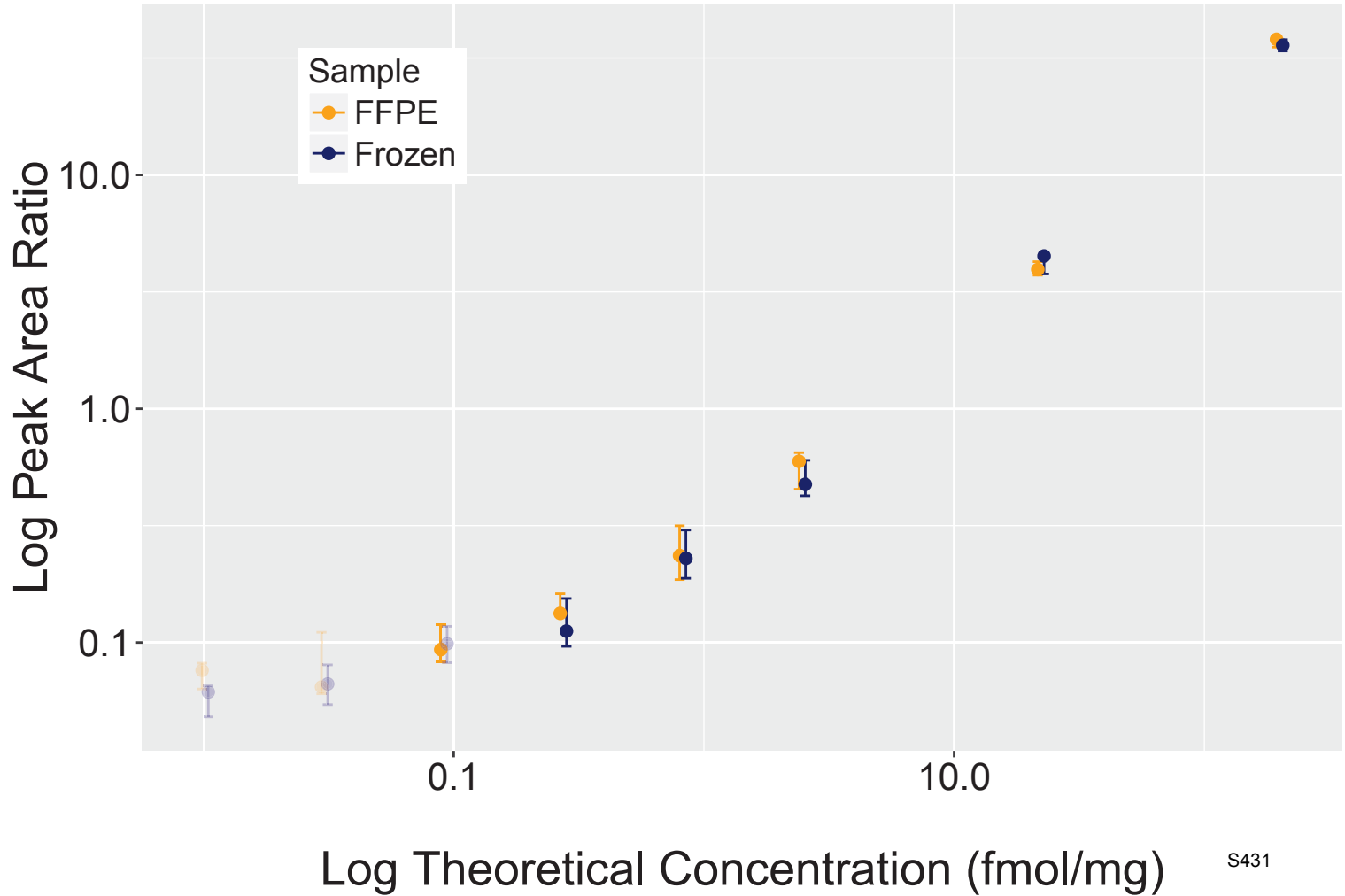
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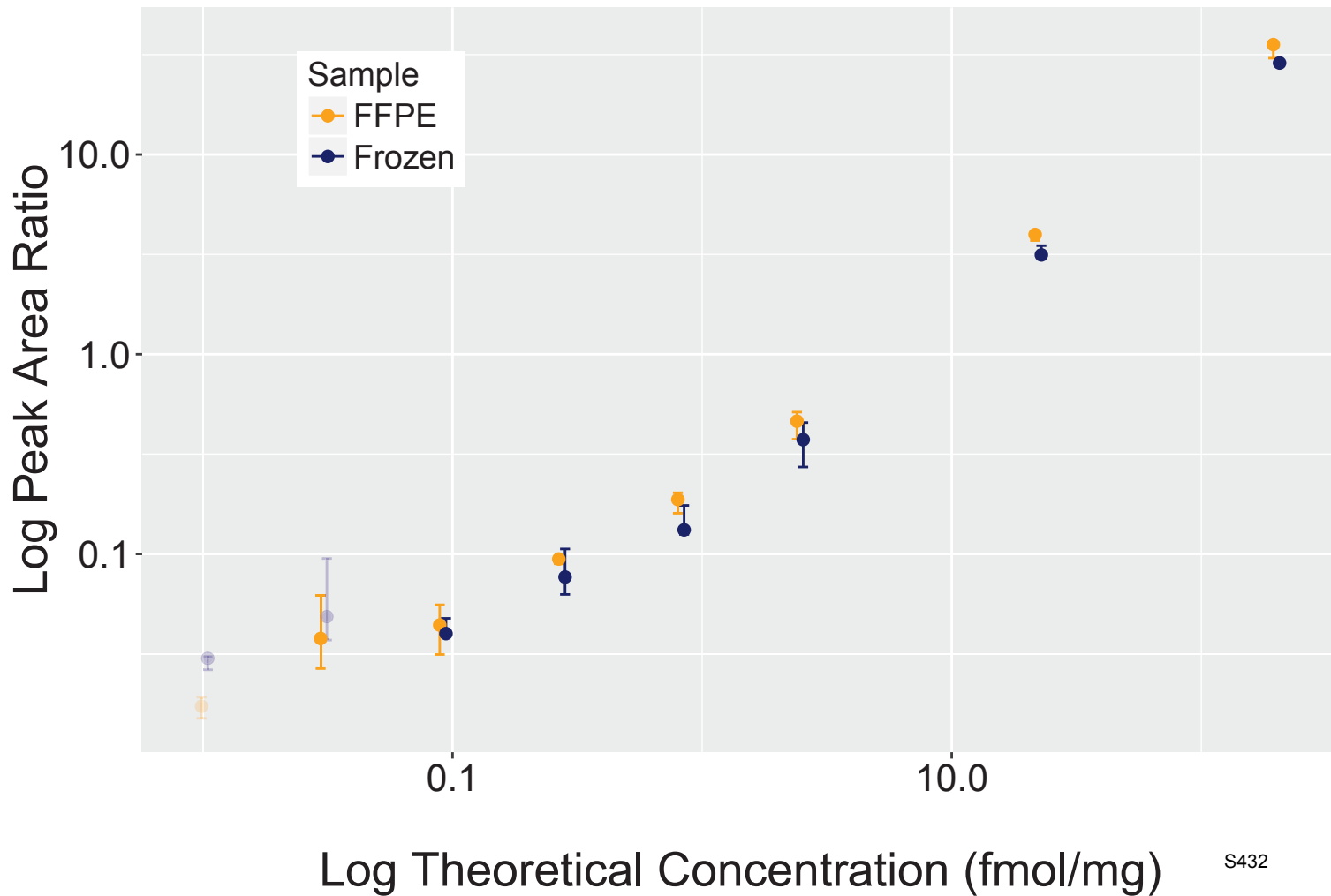
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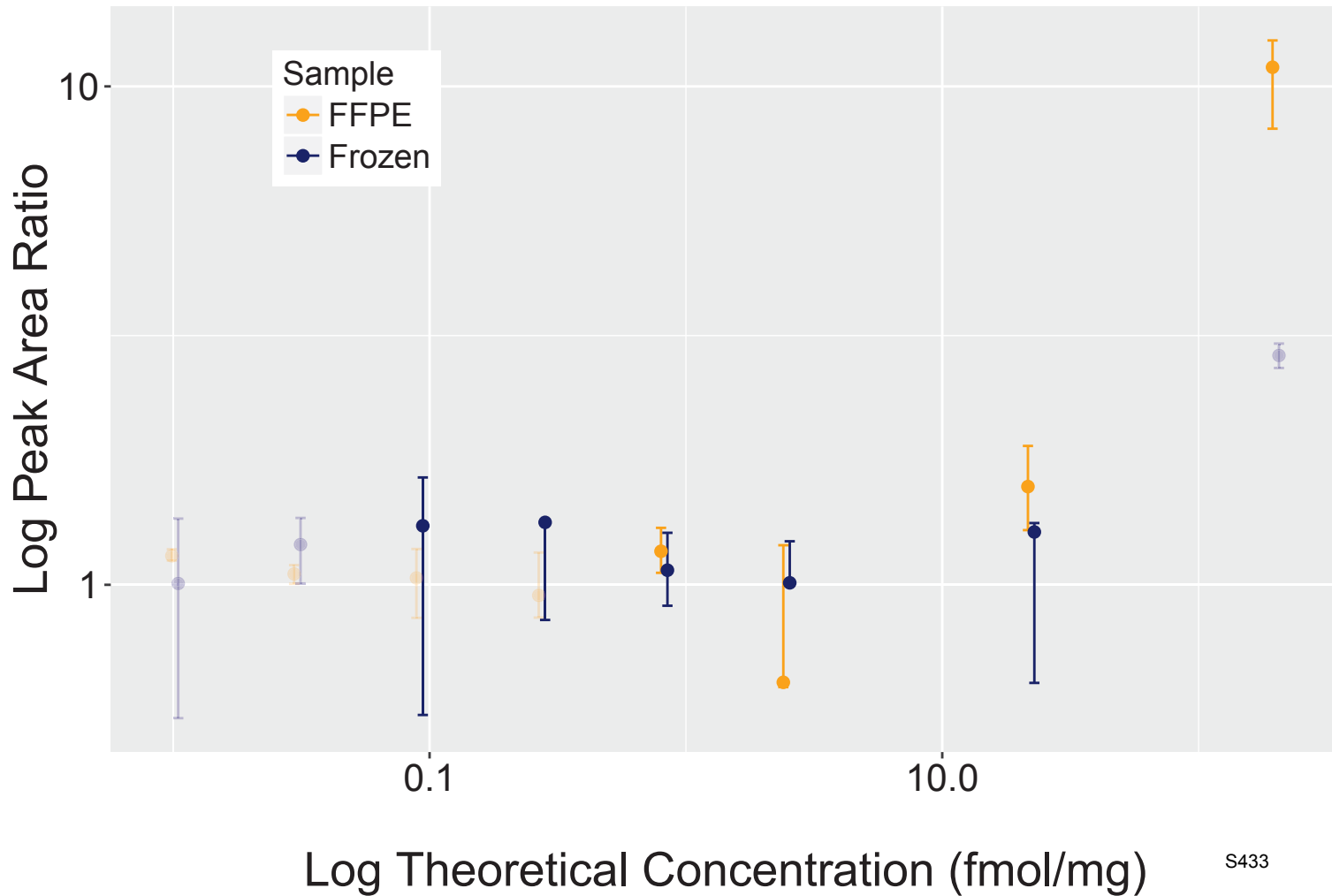
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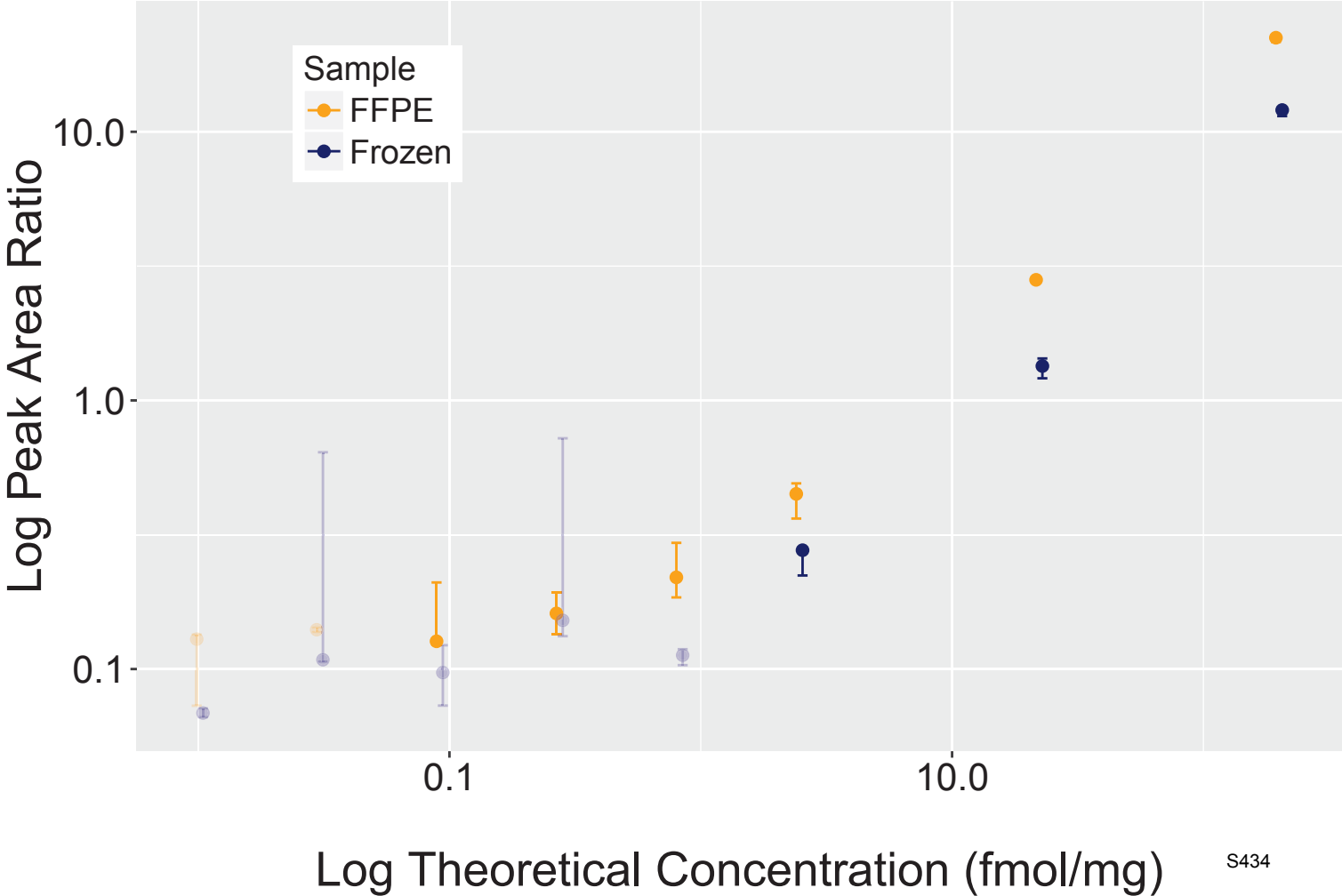
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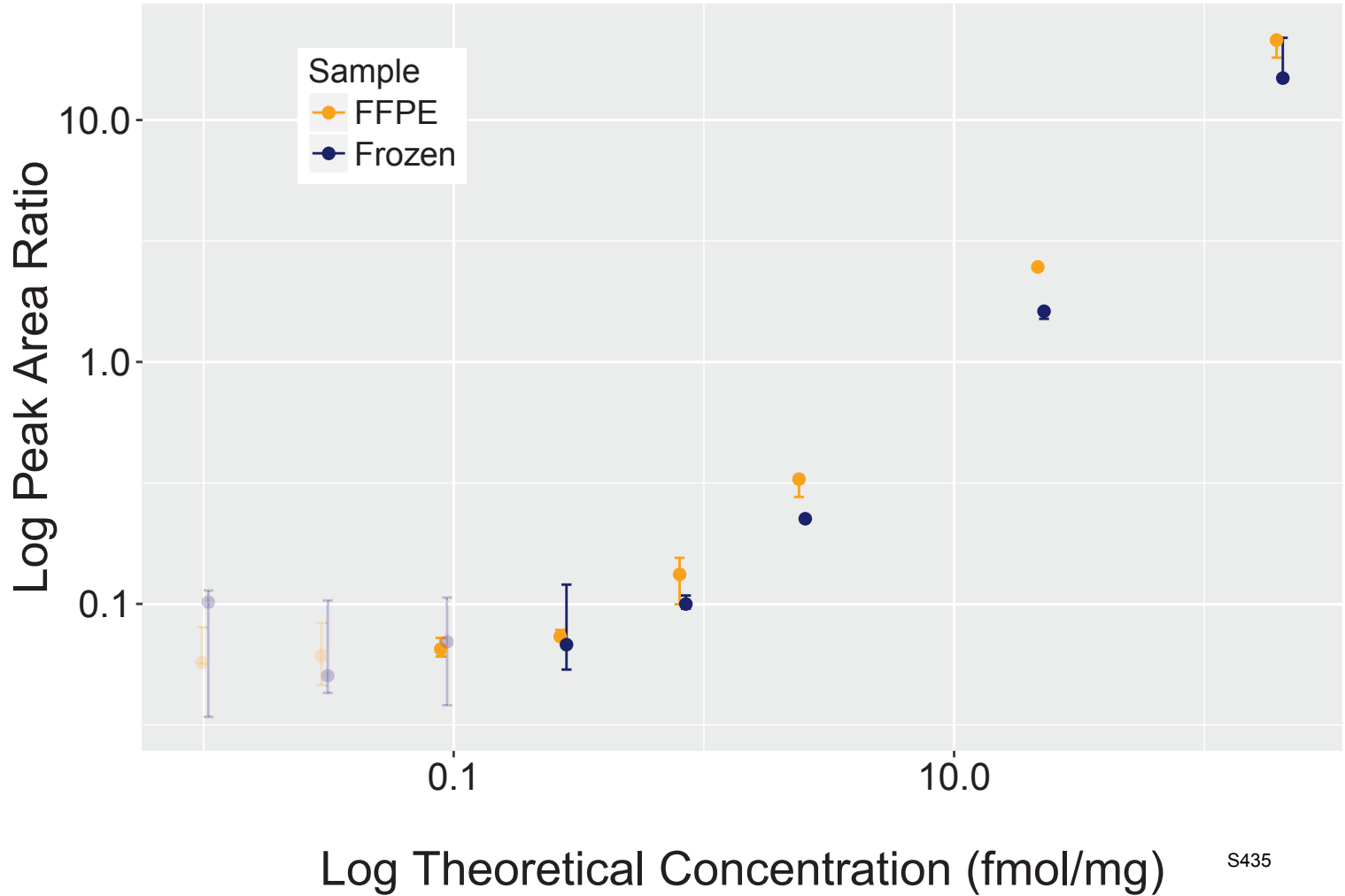
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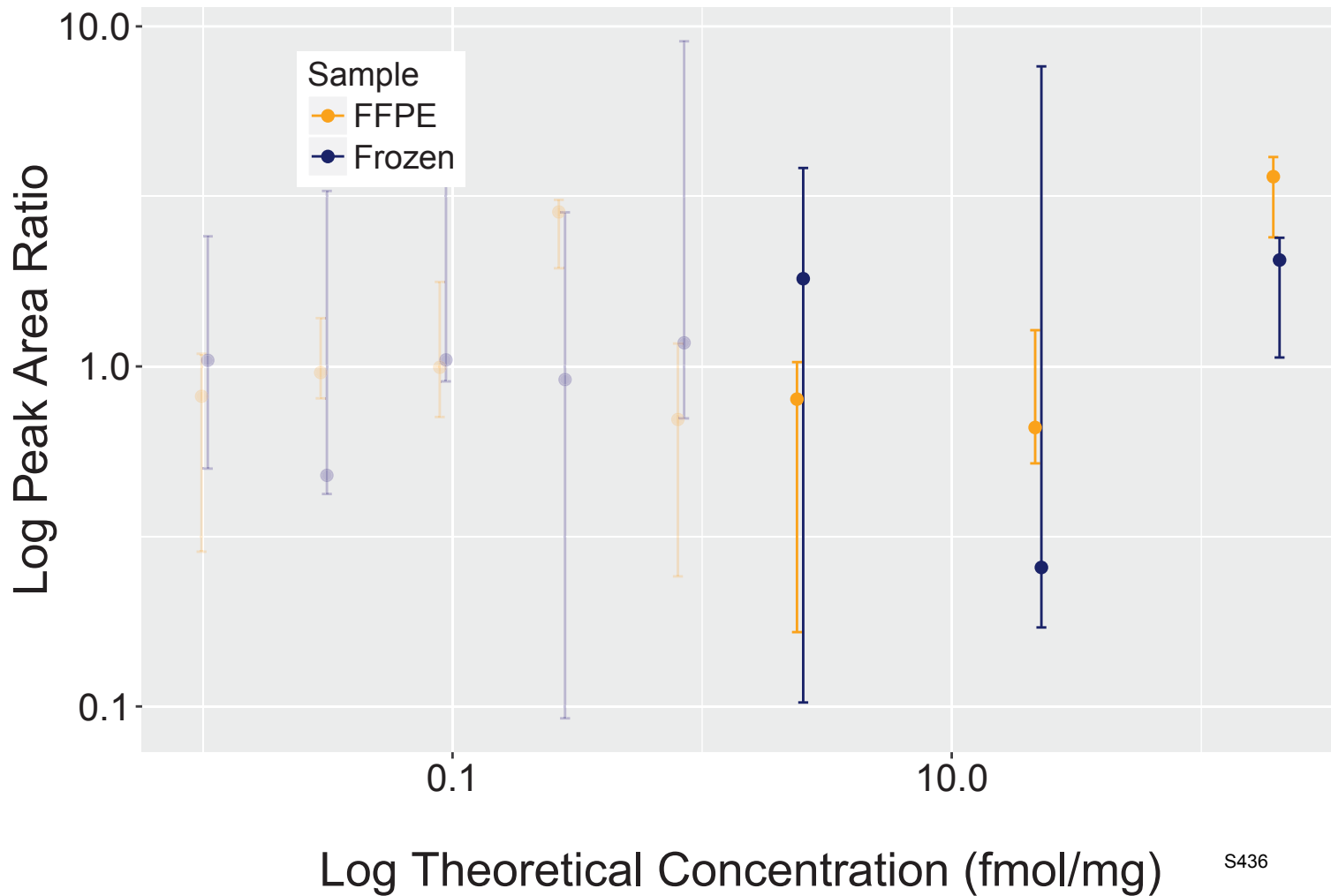
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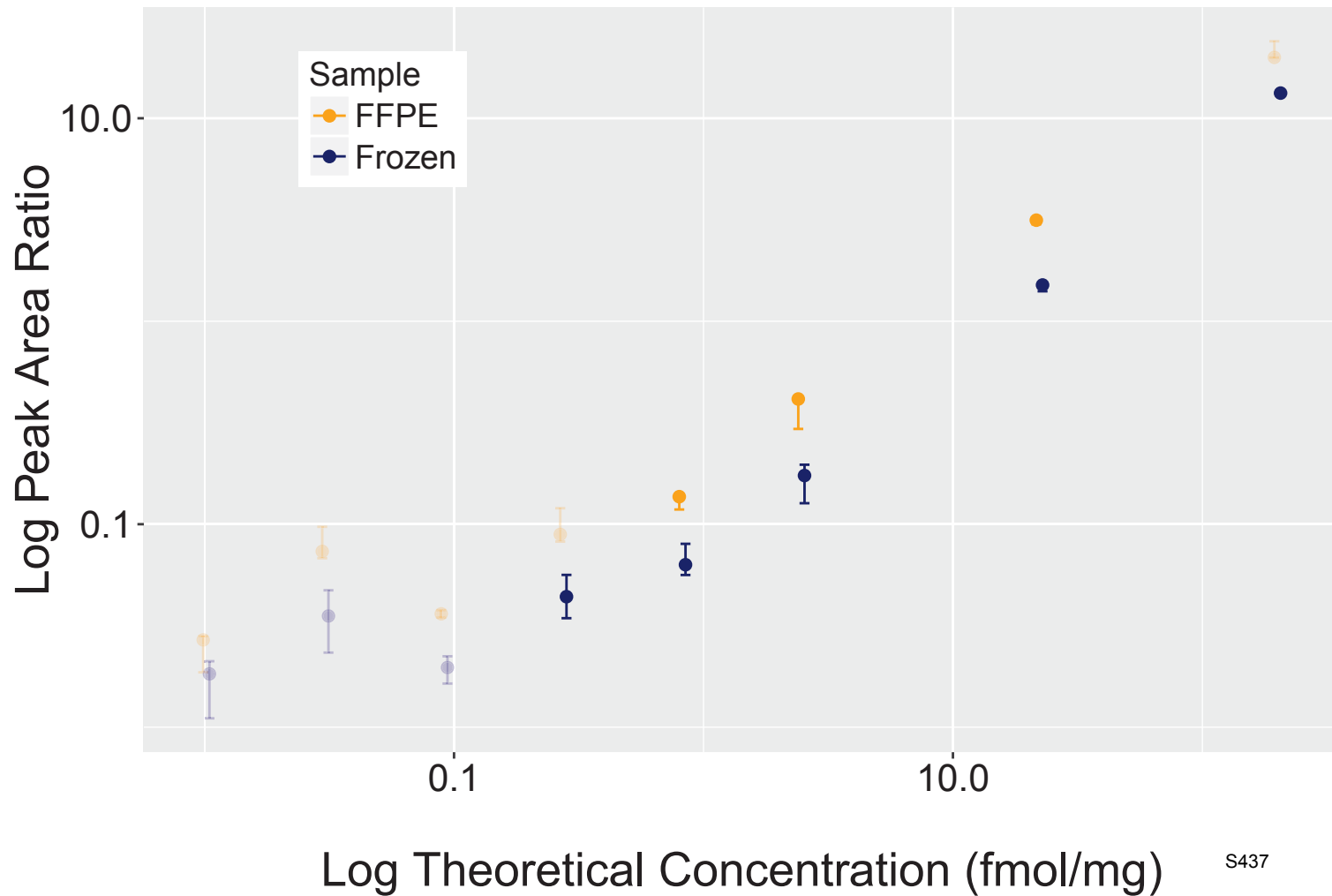
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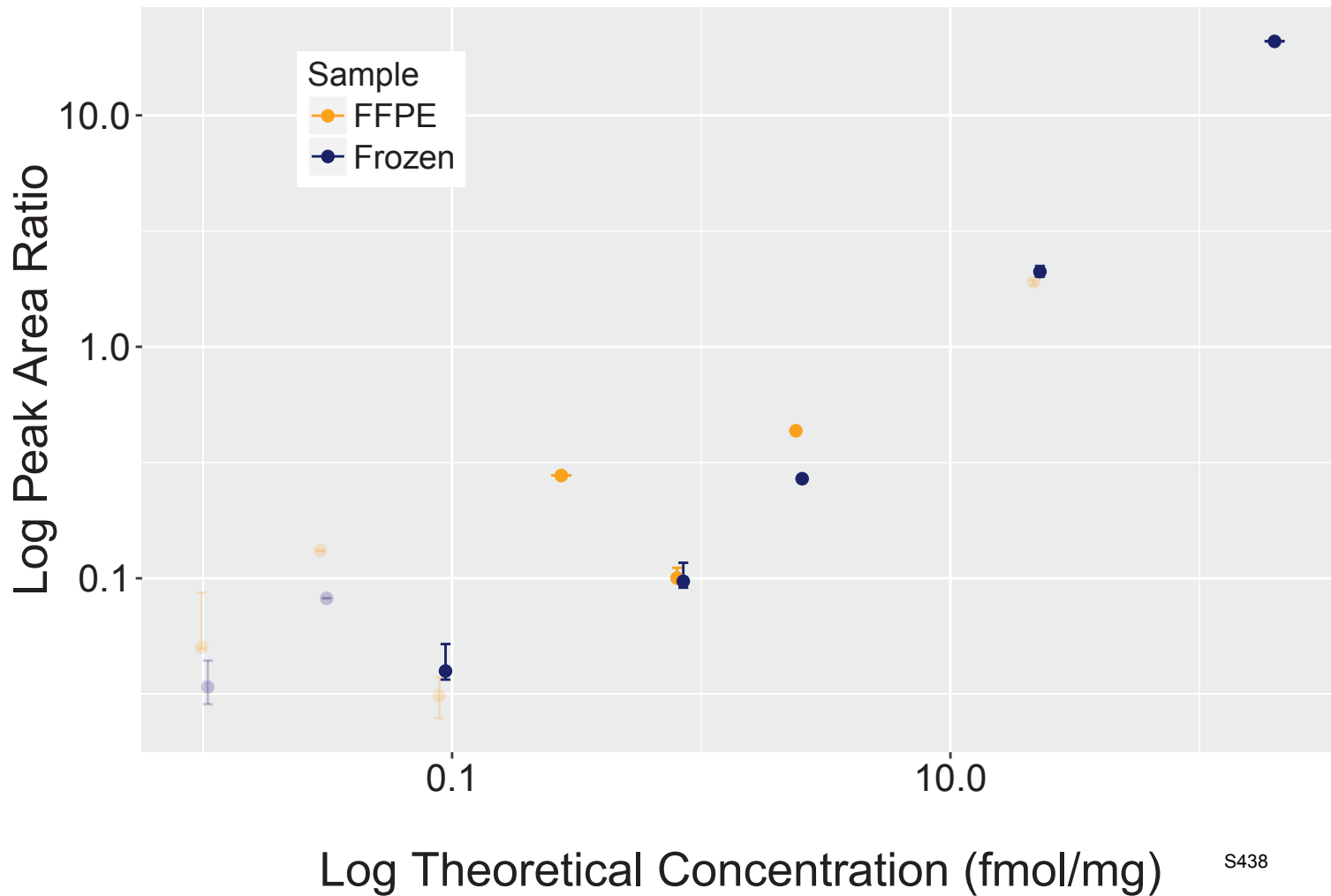
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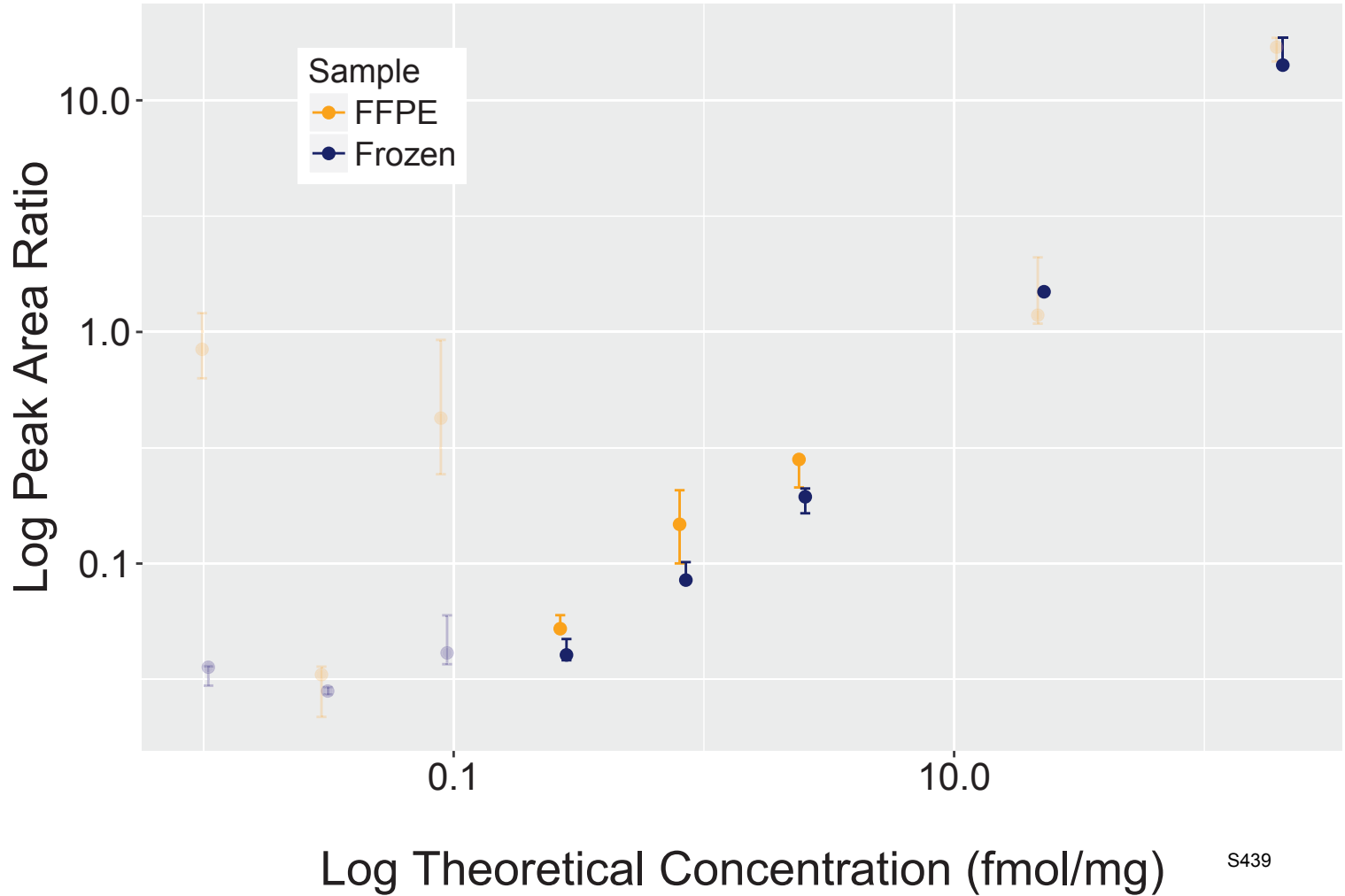
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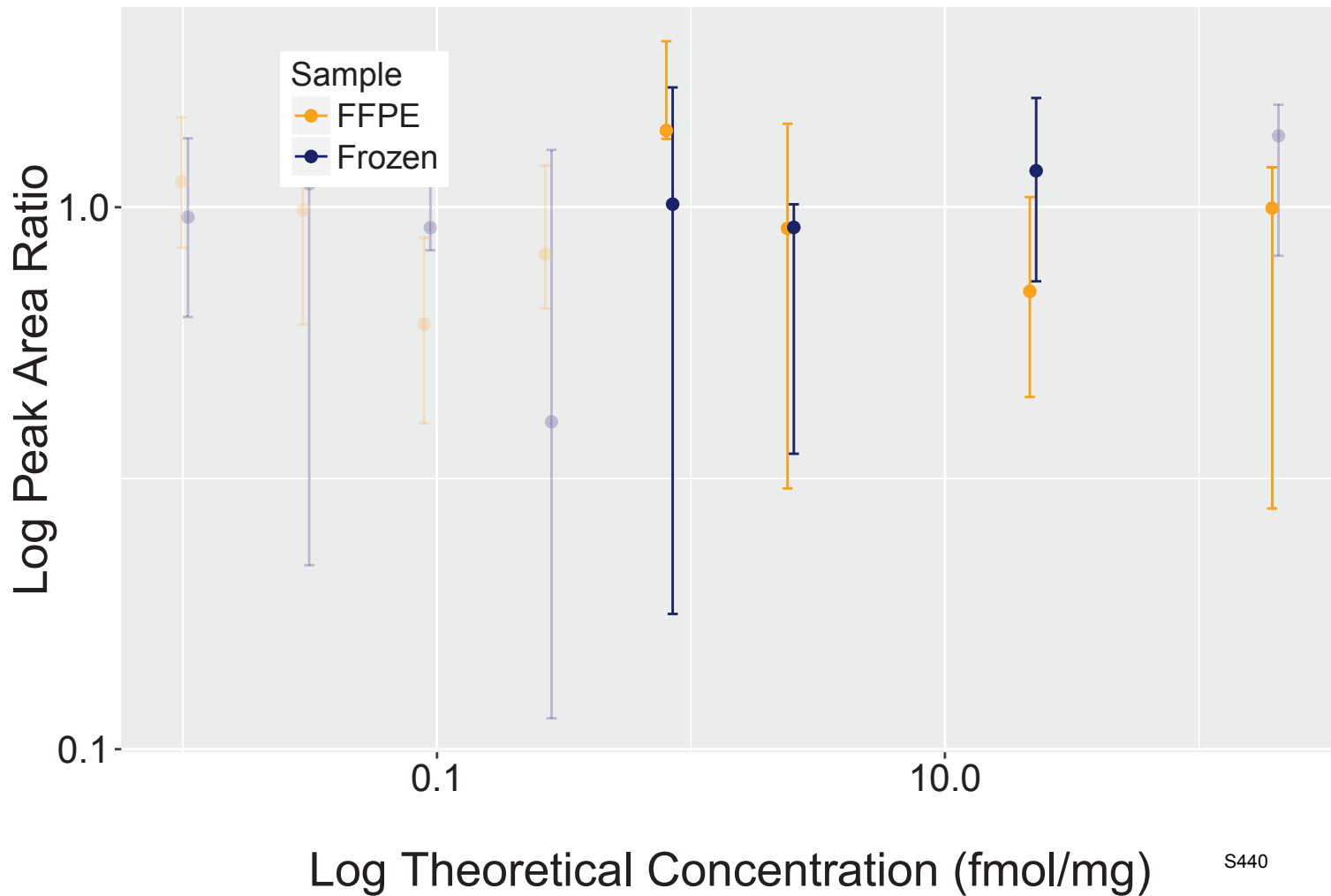
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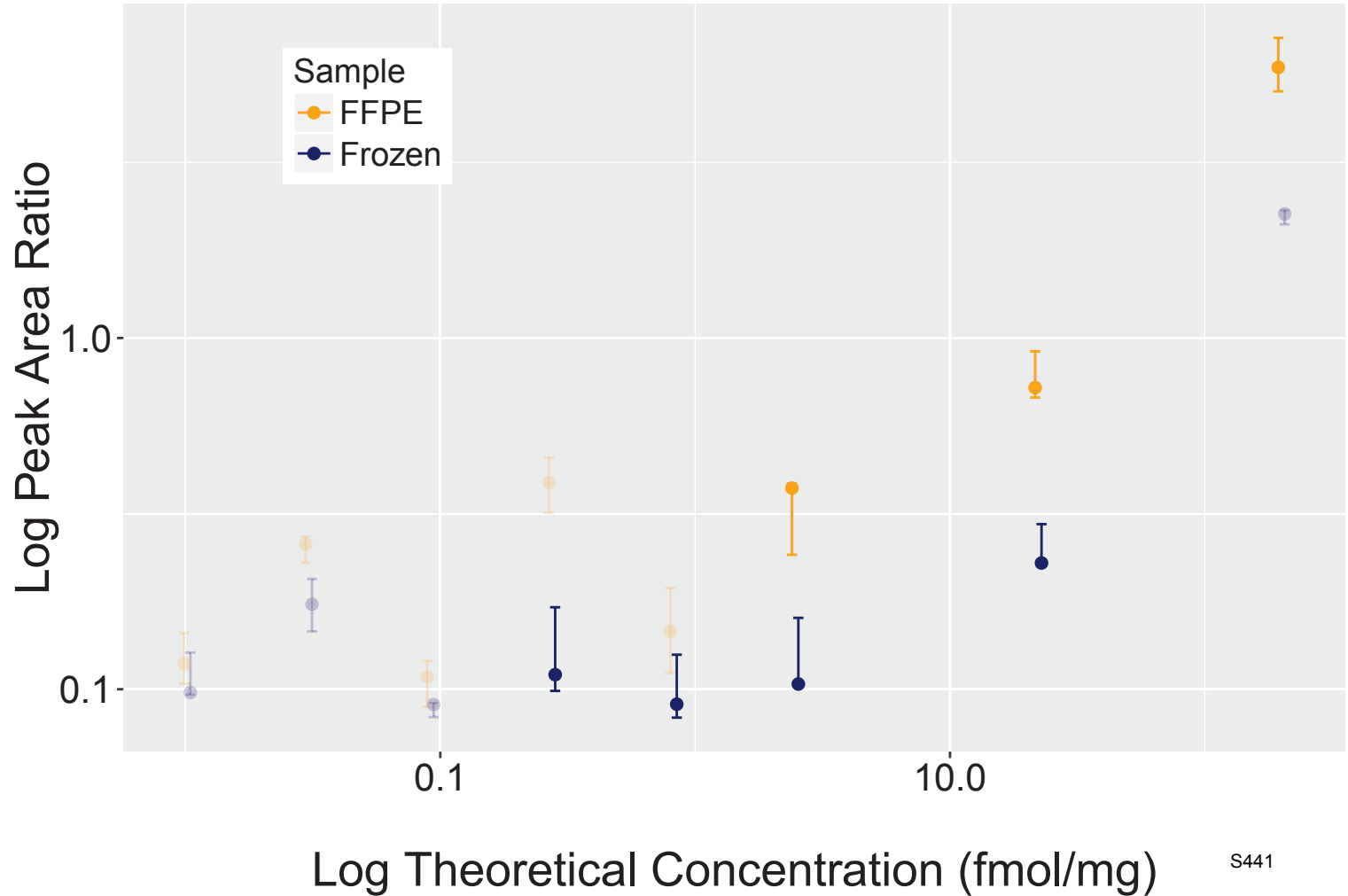
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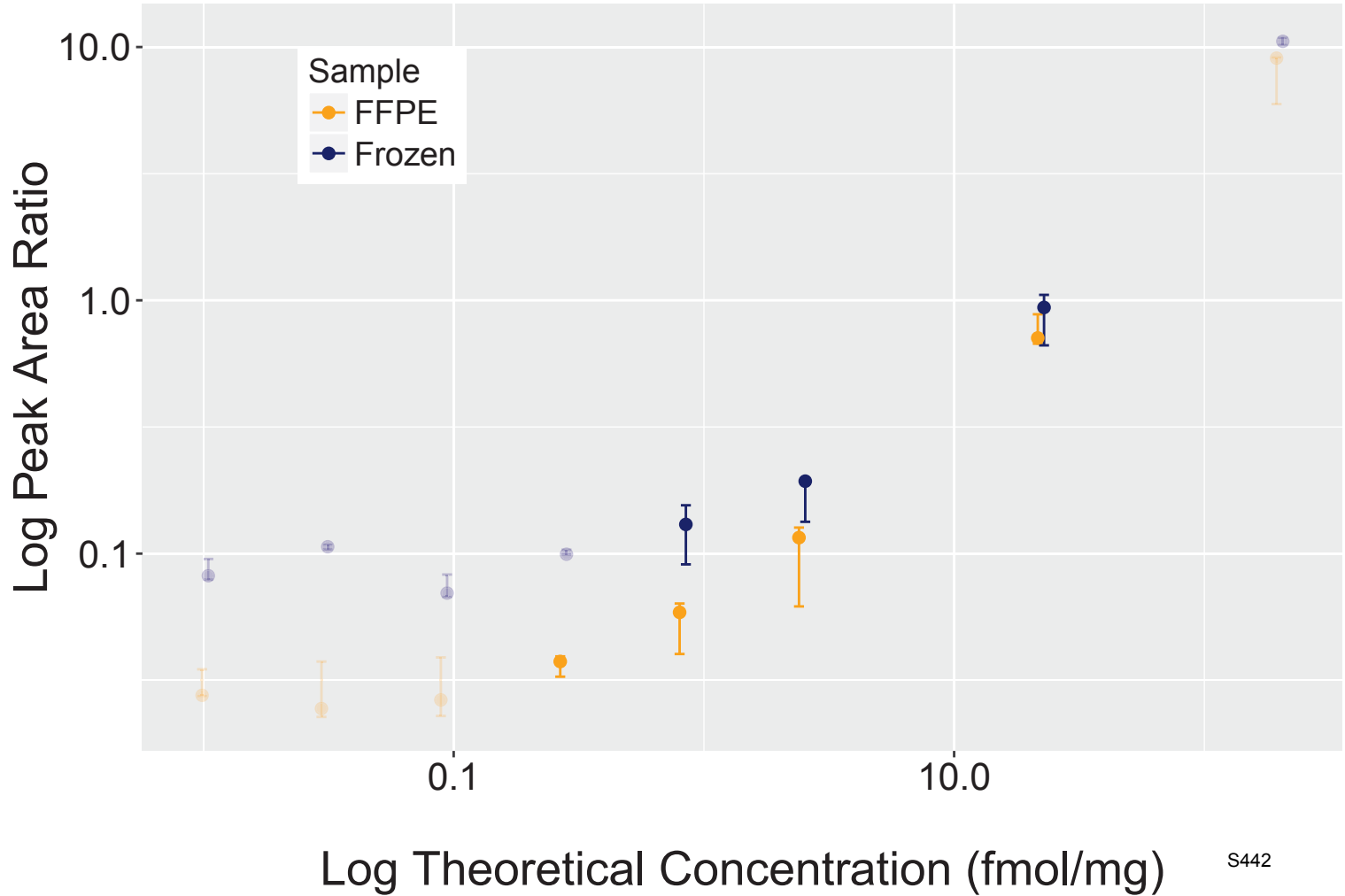
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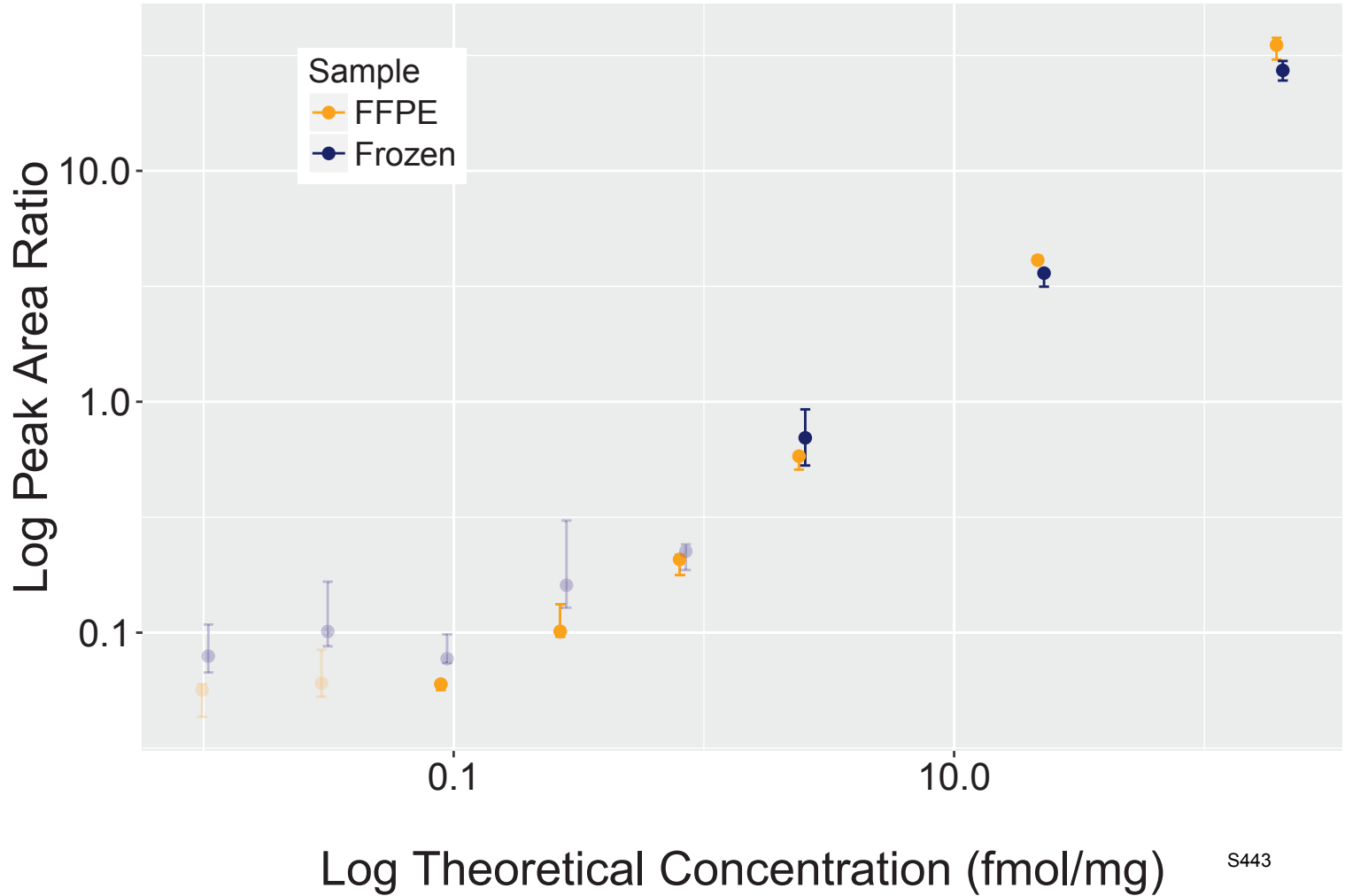
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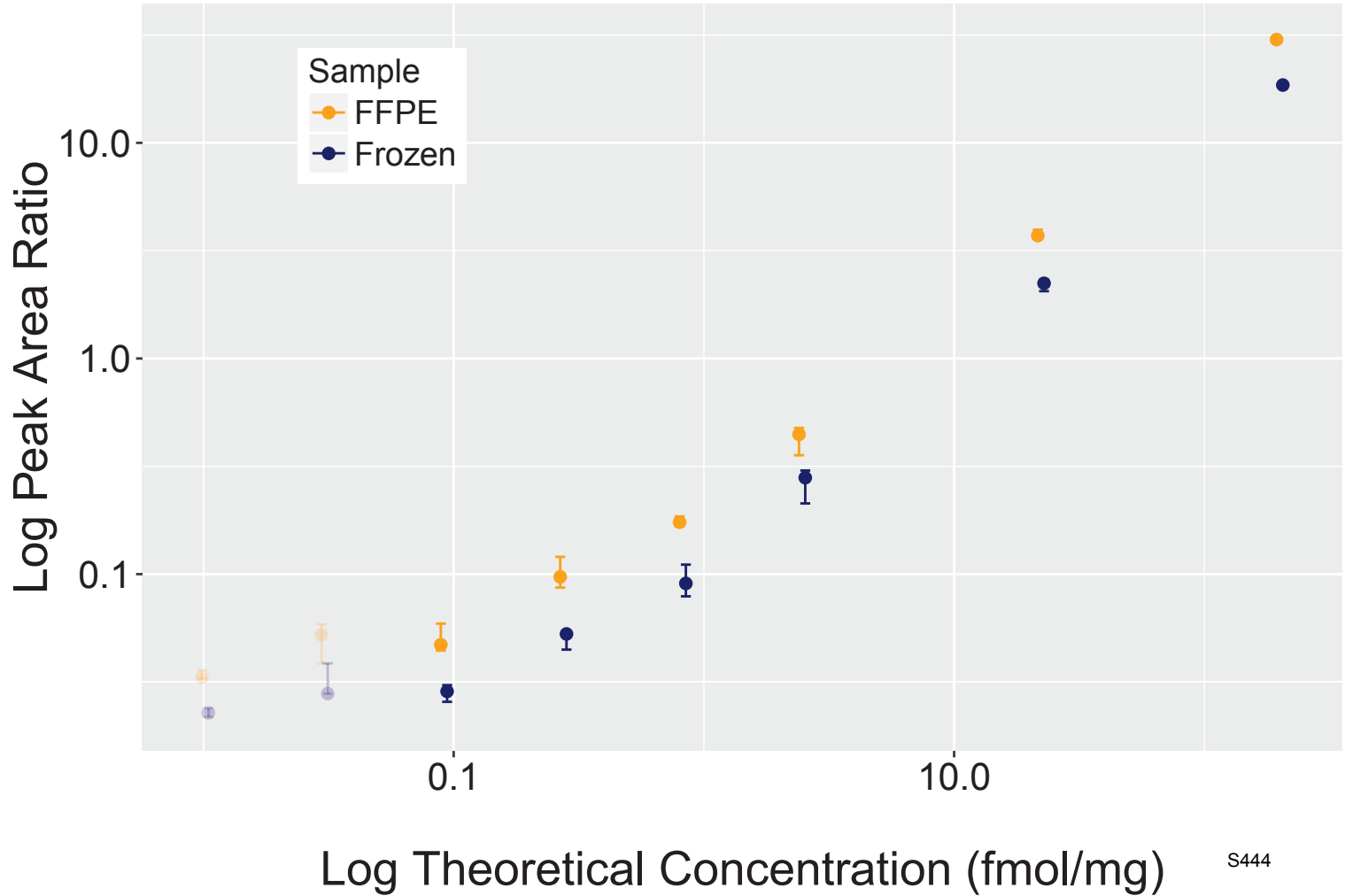
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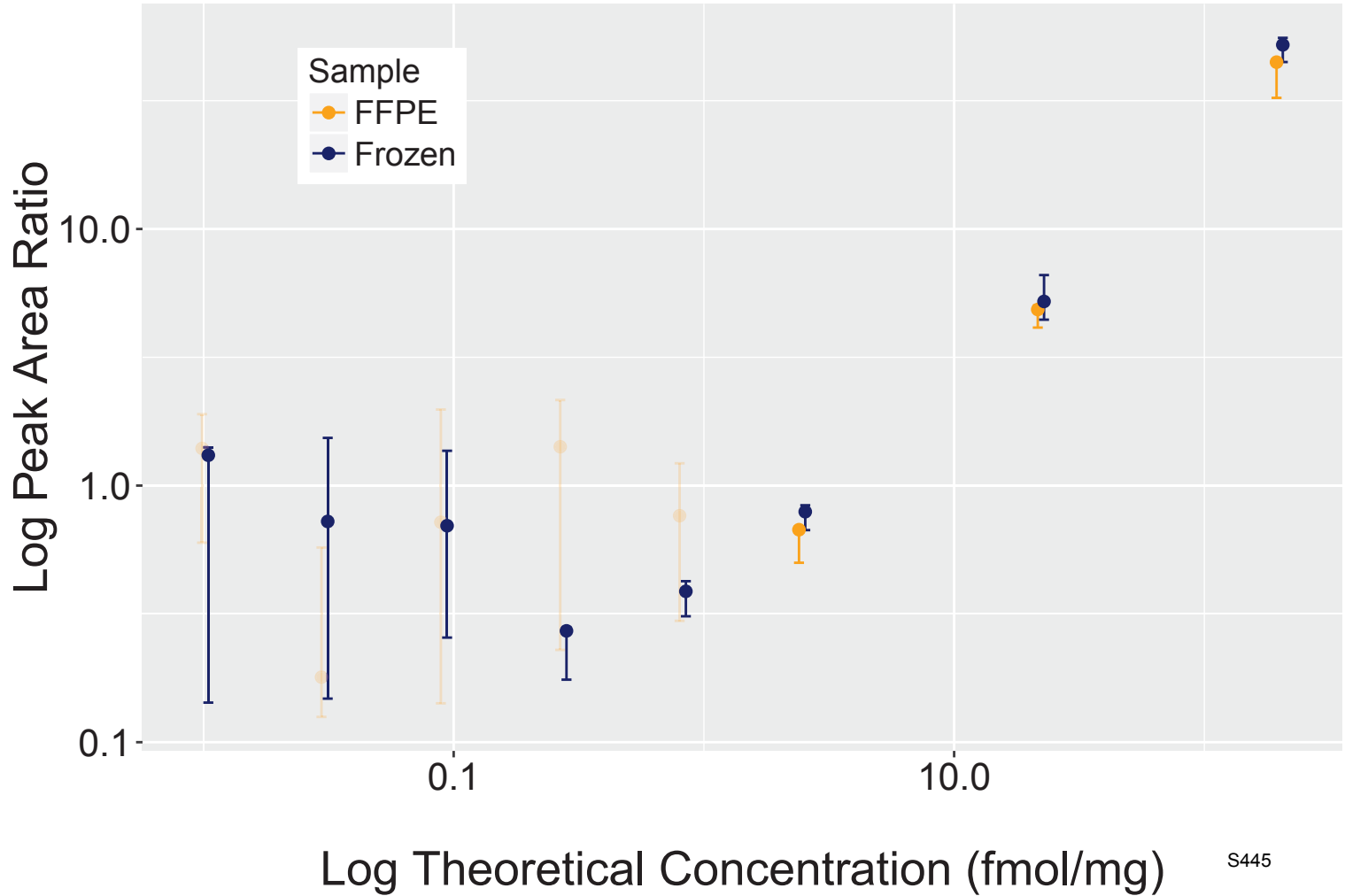
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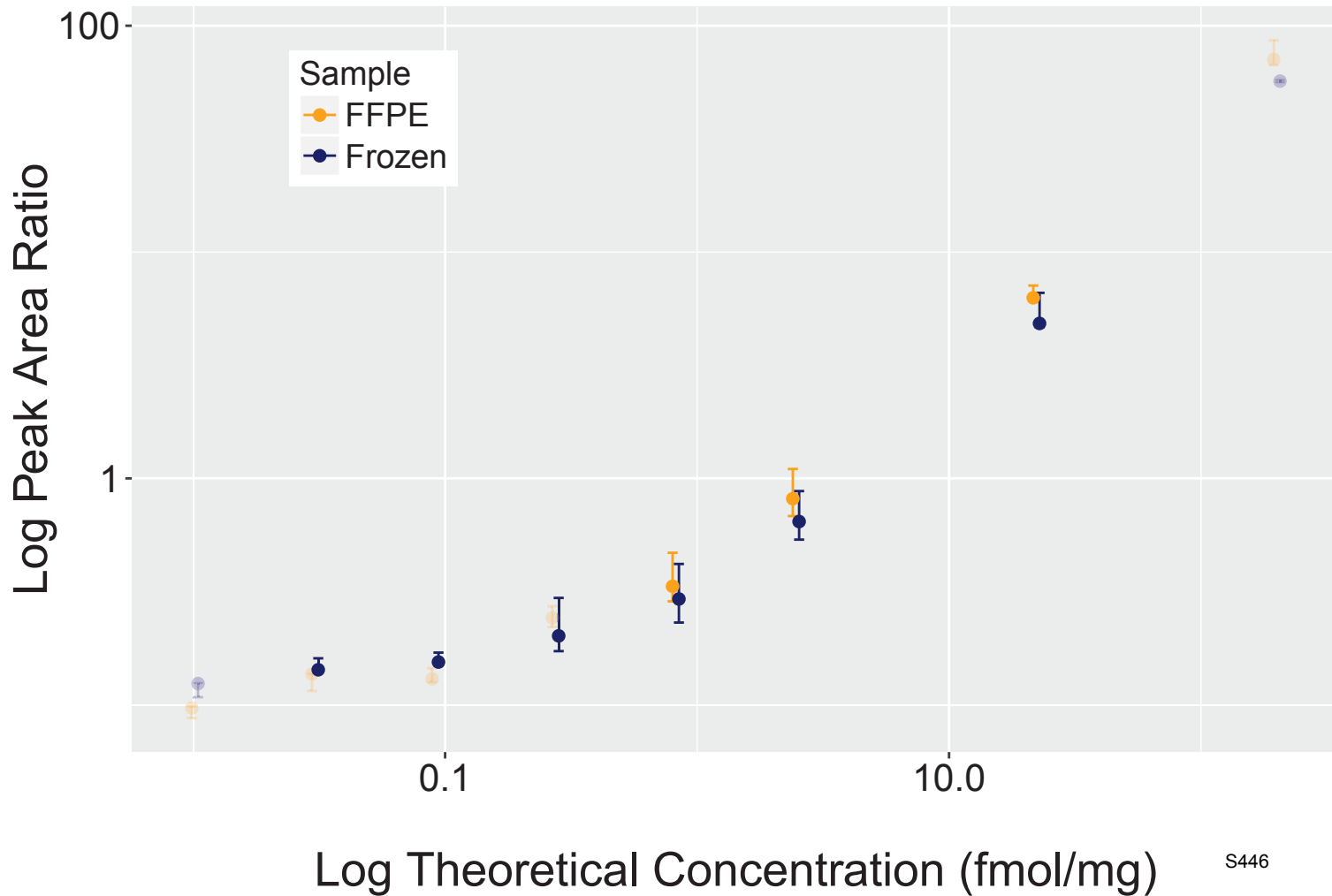
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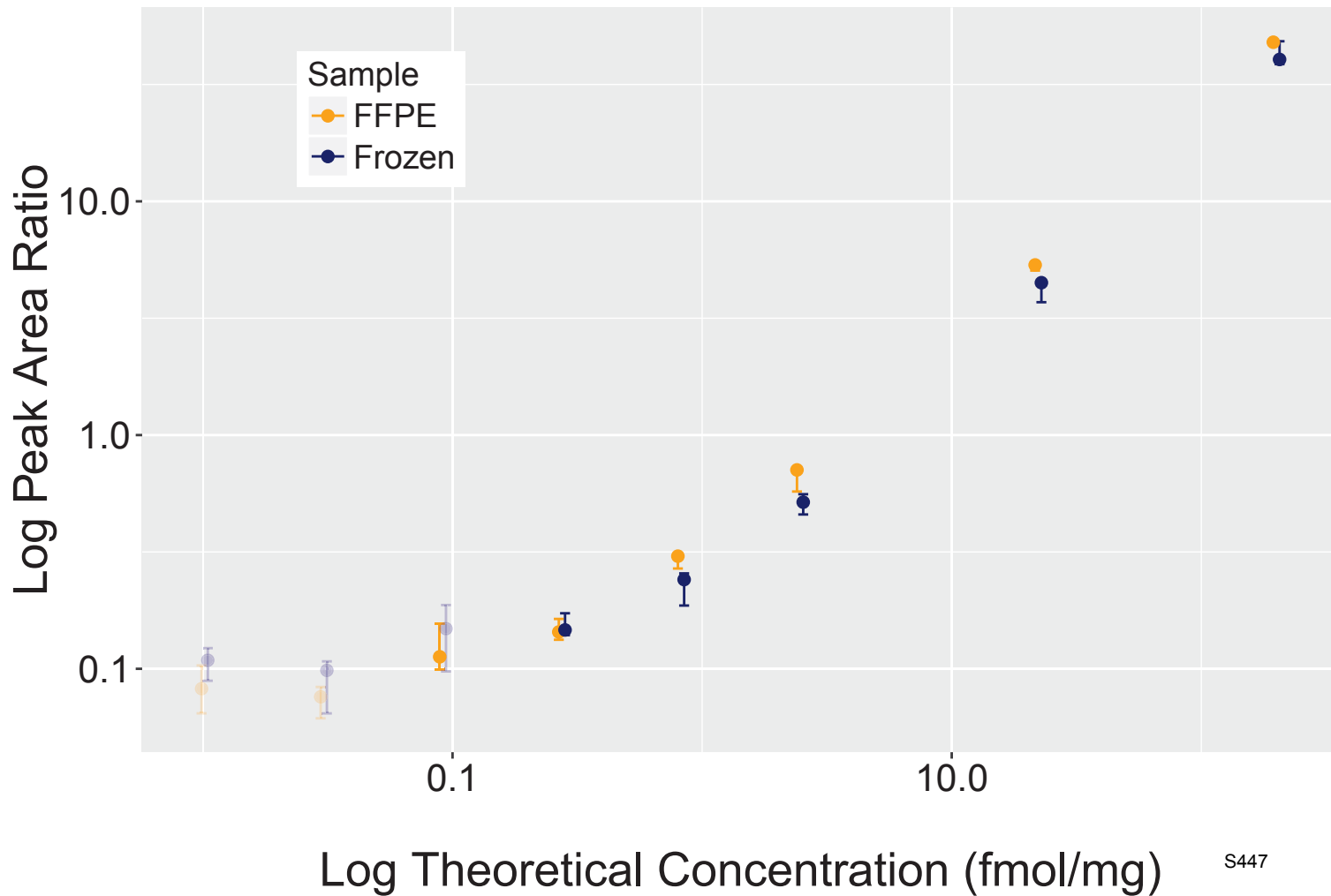
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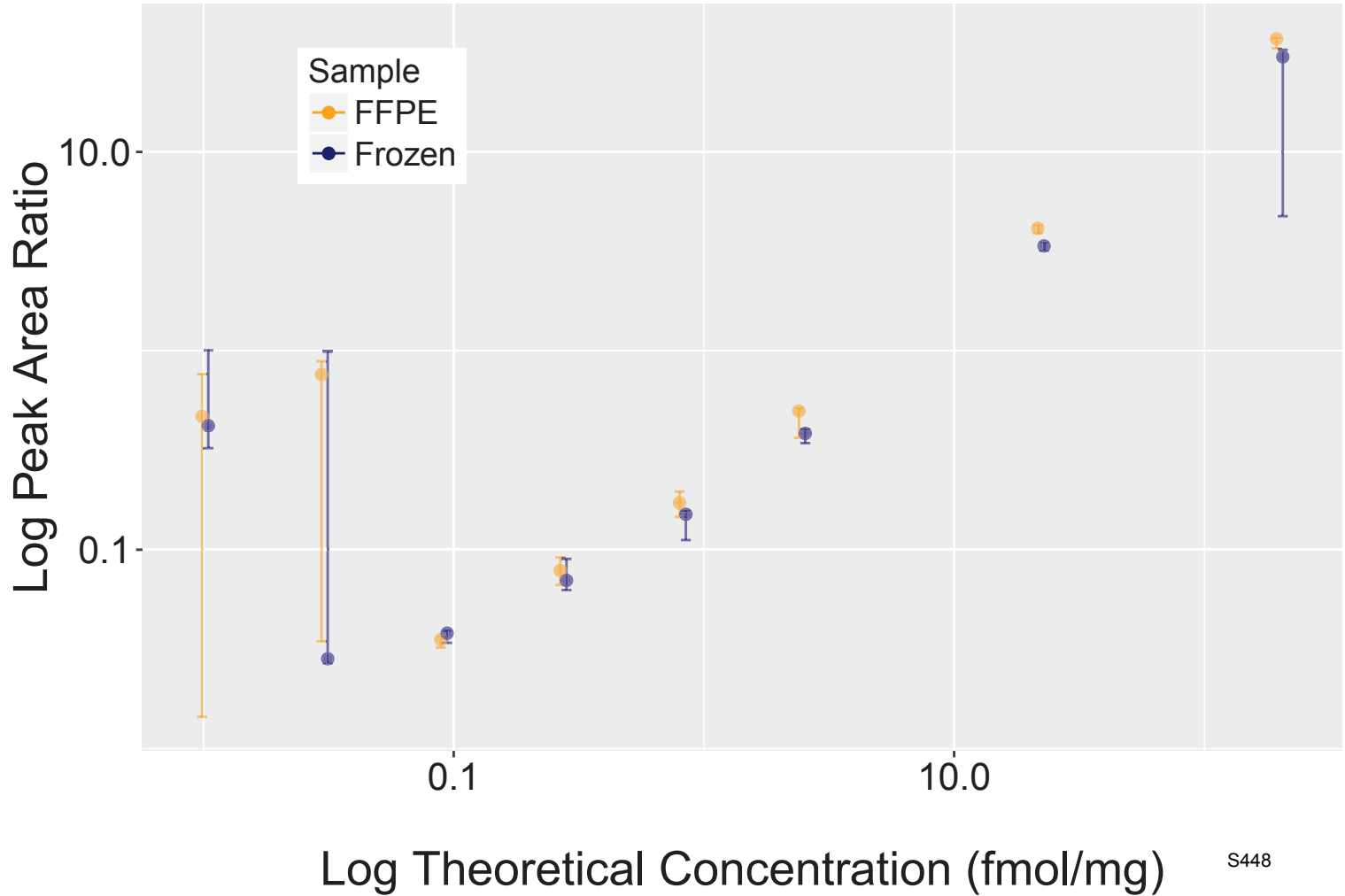
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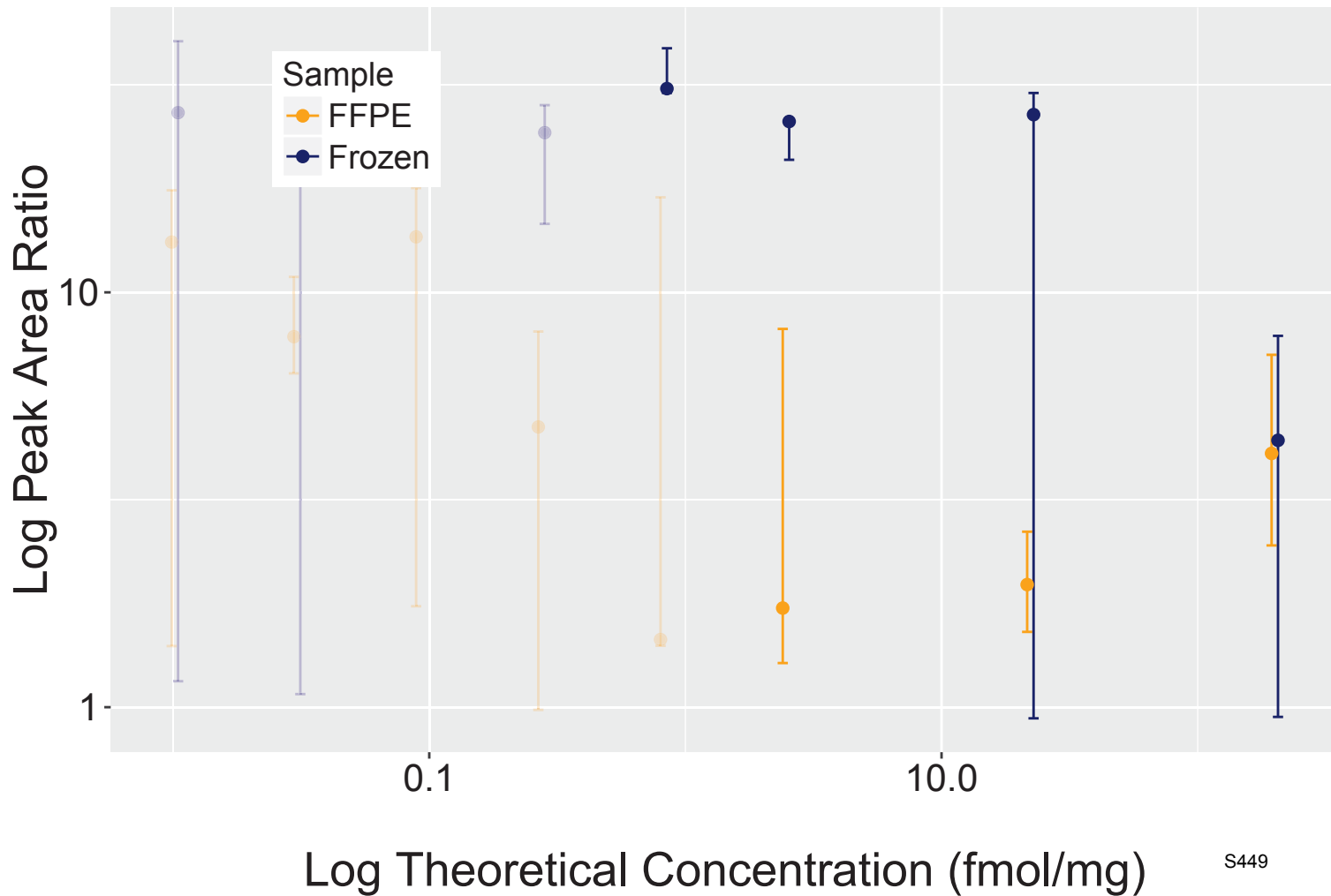
Analyte: UCHL1.NEAIQAAHDAVAQEGQC[+57]R



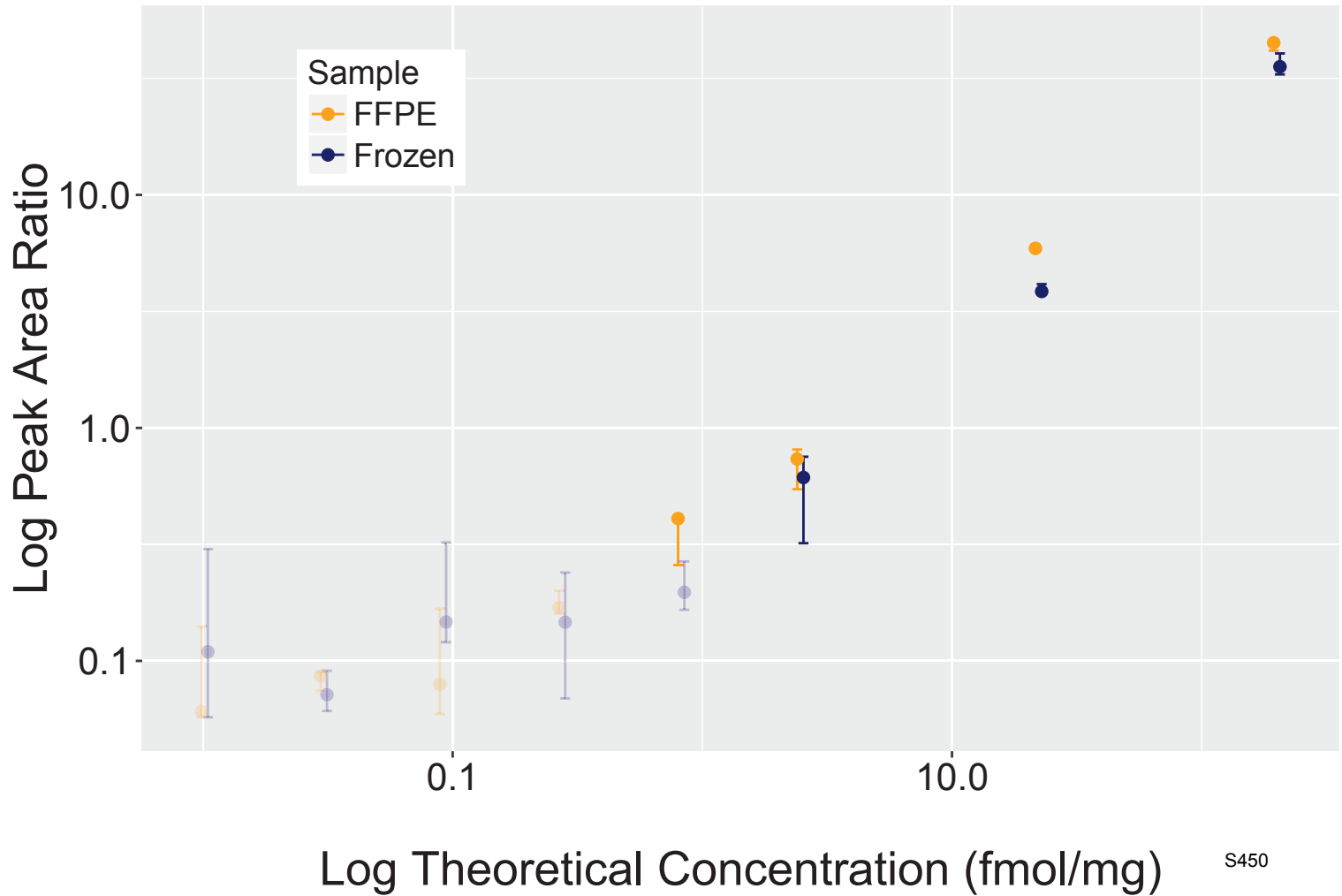
Analyte: TARS.NELSGALTGLTR



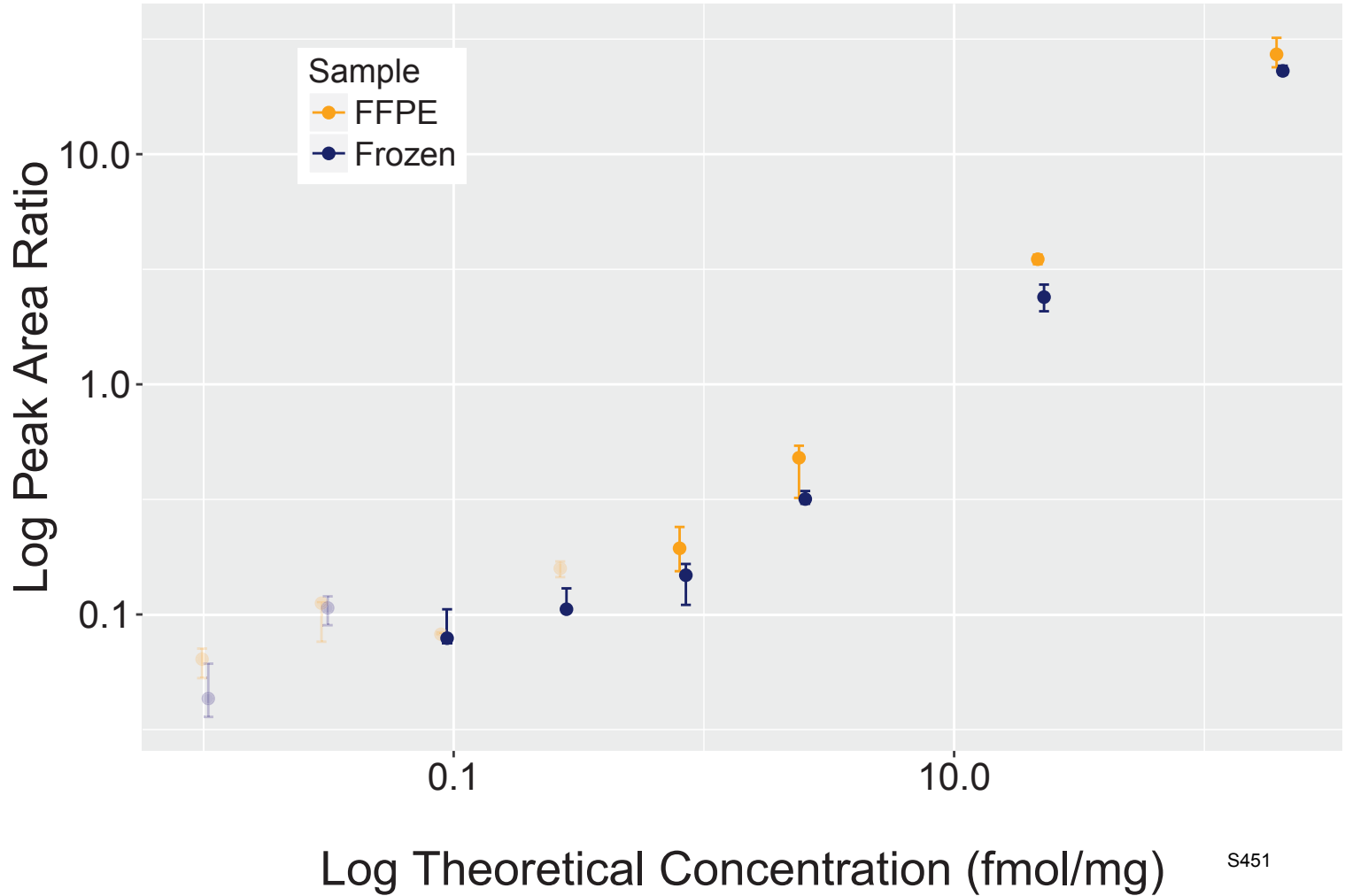
Analyte: CTSB.NGPVEGAFSVYSDFLLYK



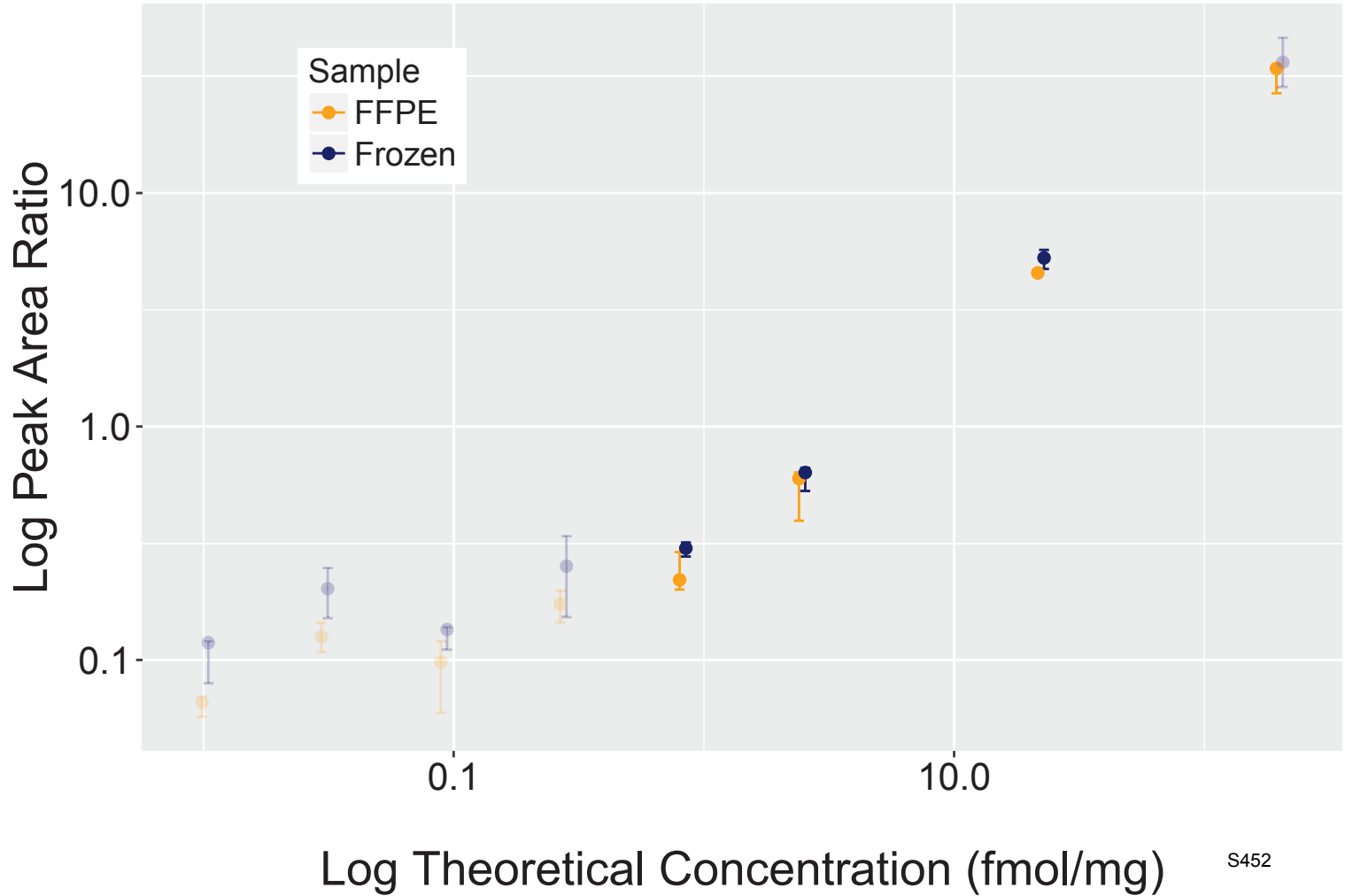
Analyte: UBA1.NGSEADIDEGLYSR



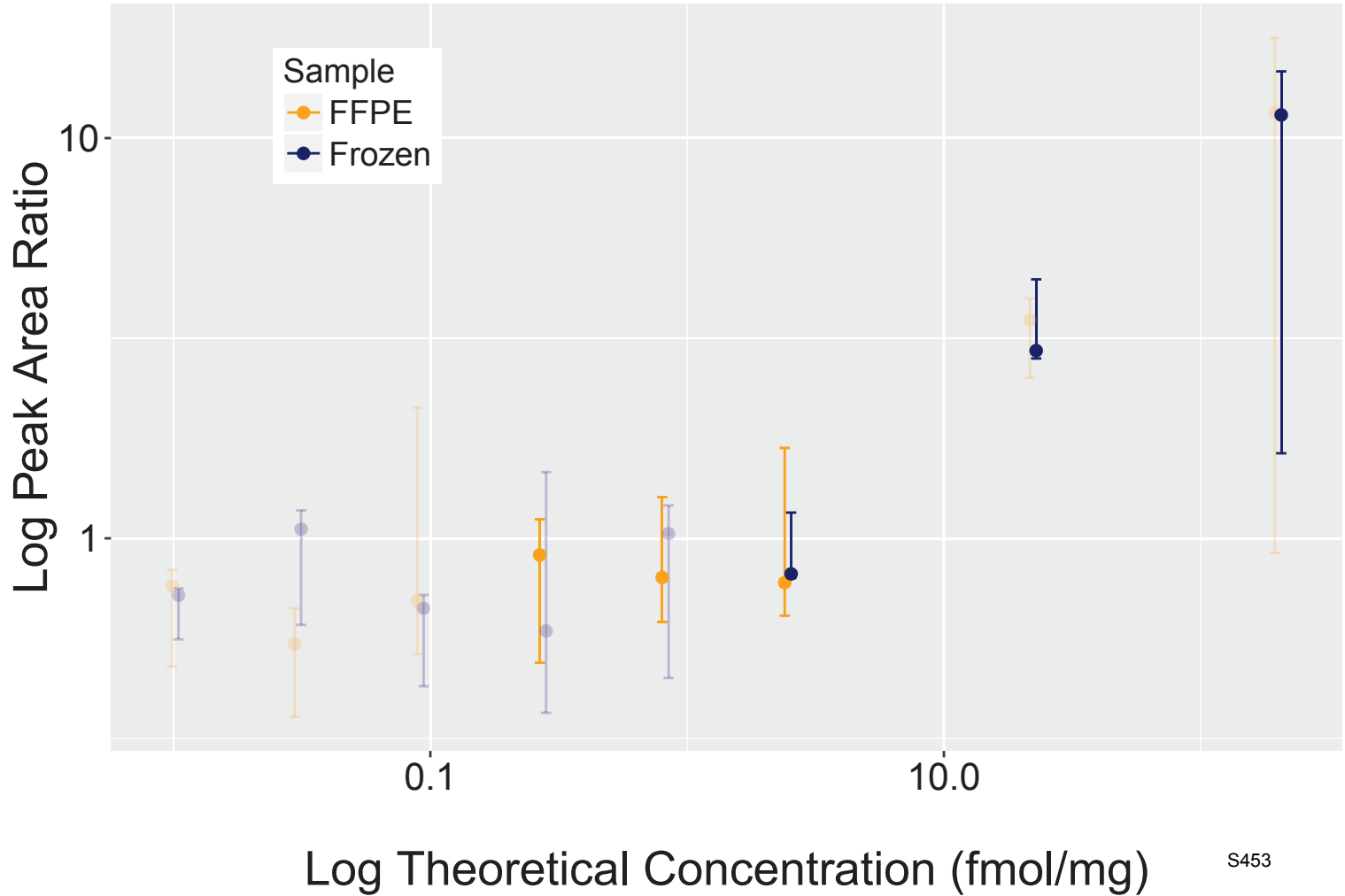
Analyte: RPN1.NIEIDSPYEISR



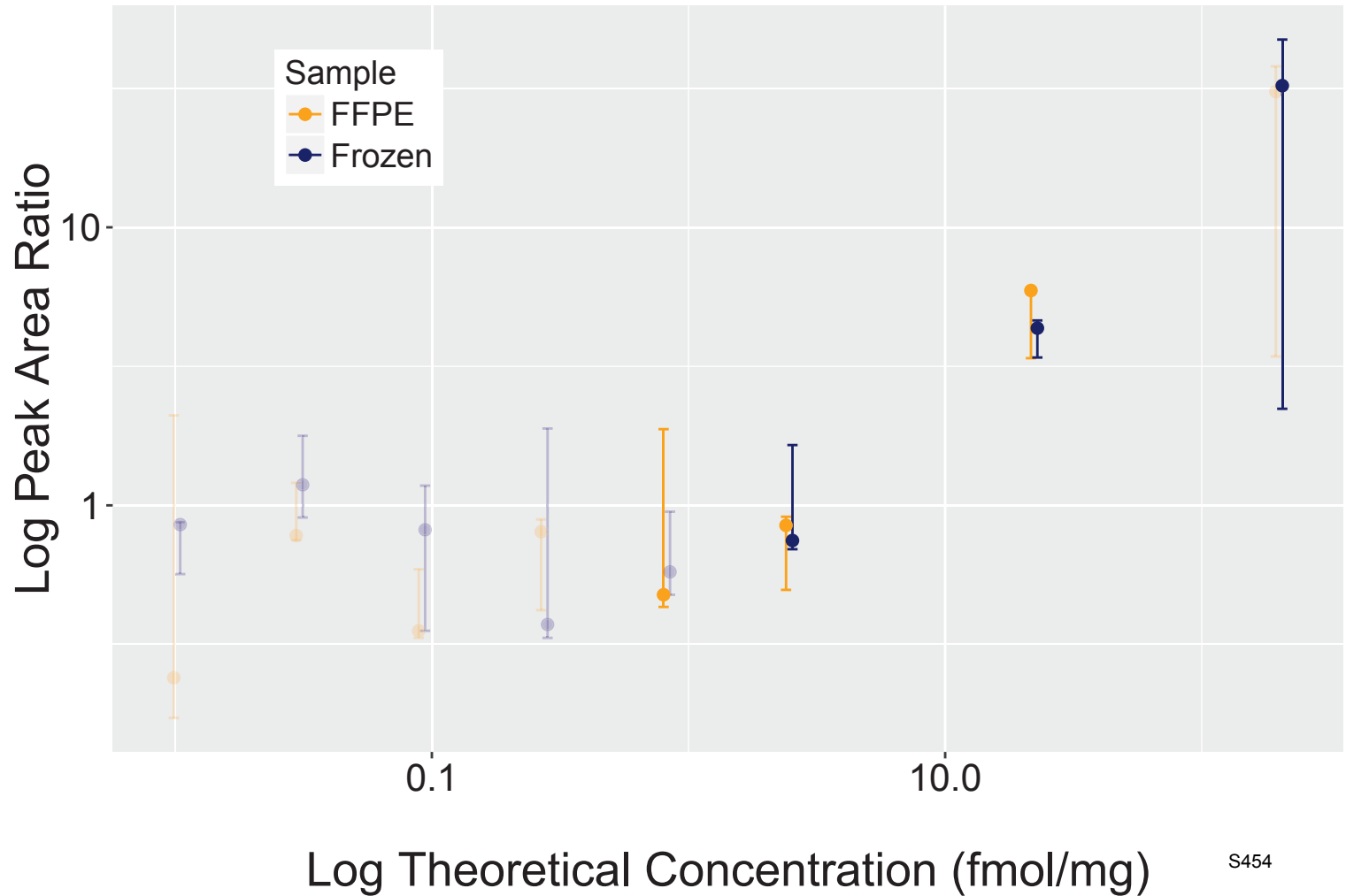
Analyte: SEC22B.NLGSINTELQDVQR



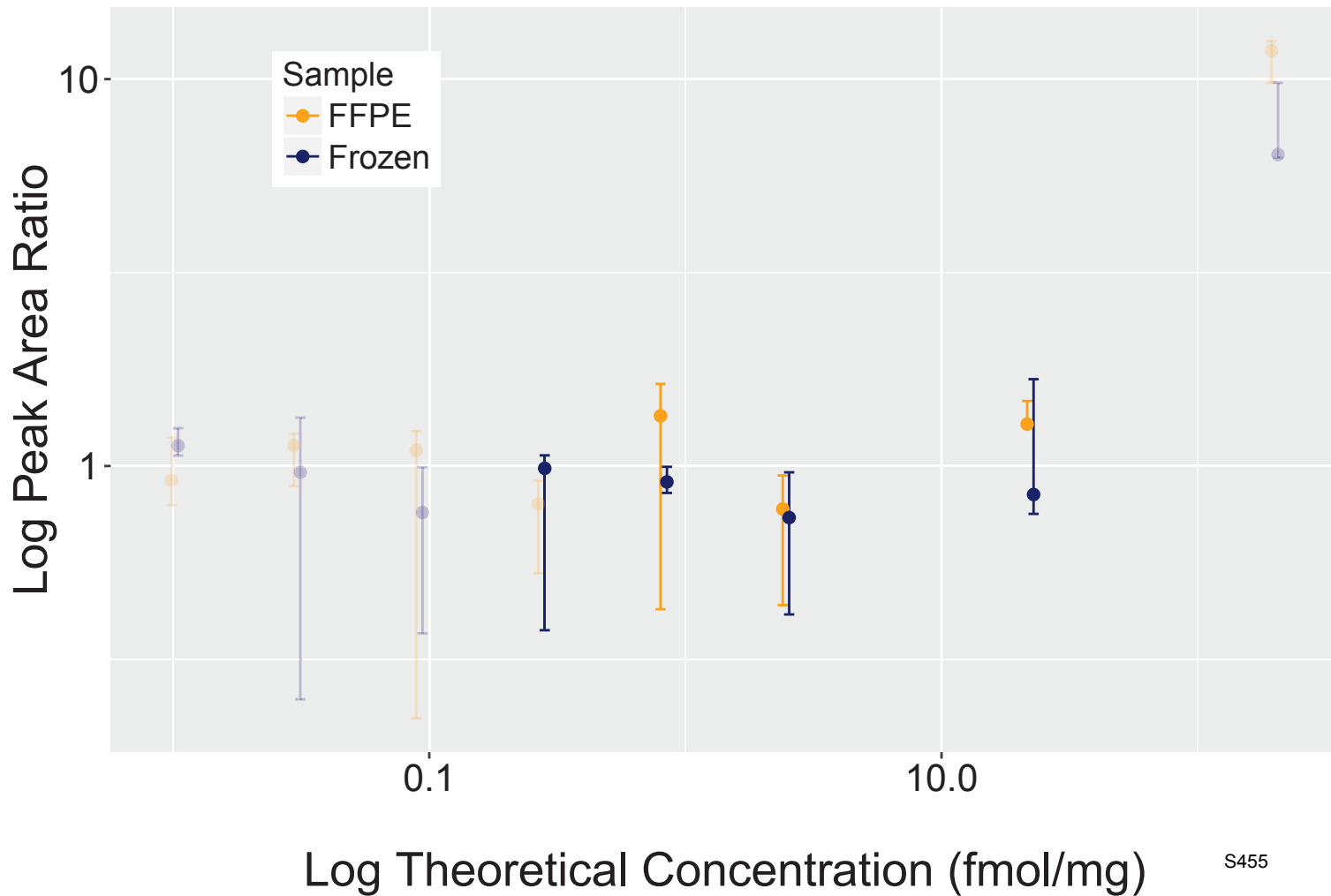
Analyte: IPO5.NLIDEDGNNQWPEGLK



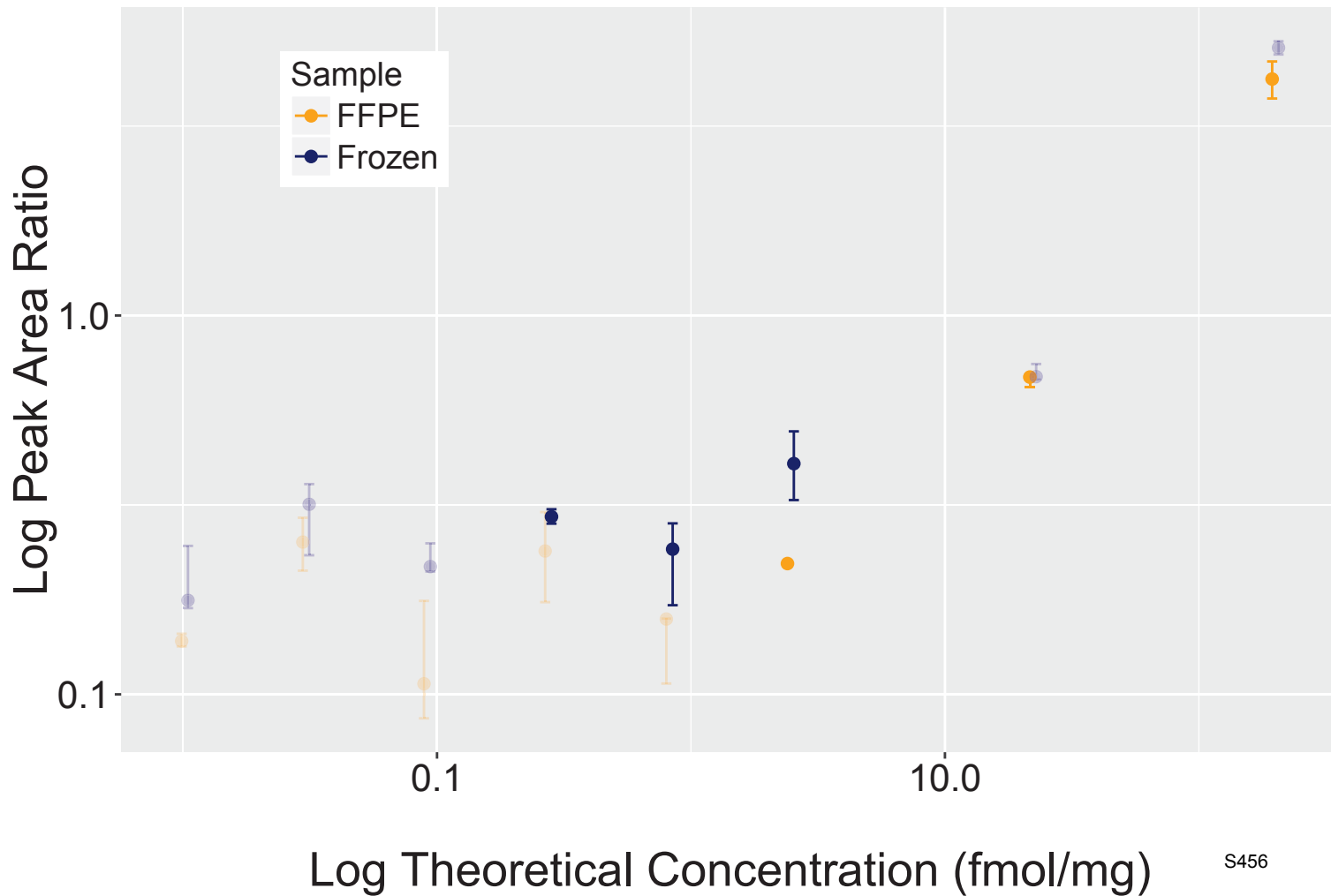
Analyte: KPNA2.NNQGTVNWSVDDIVK



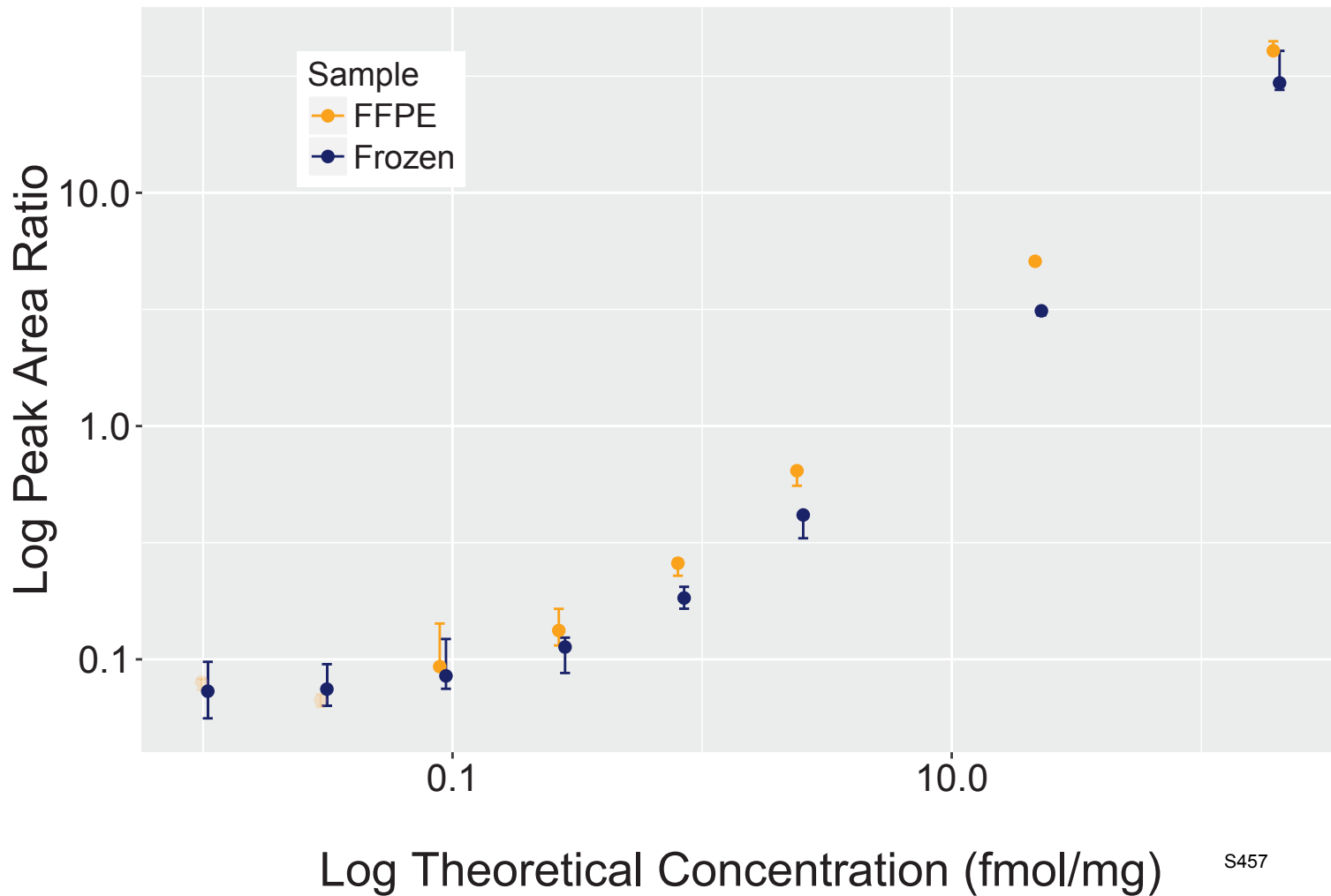
Analyte: PGD.NPELQNLLLDDFFK



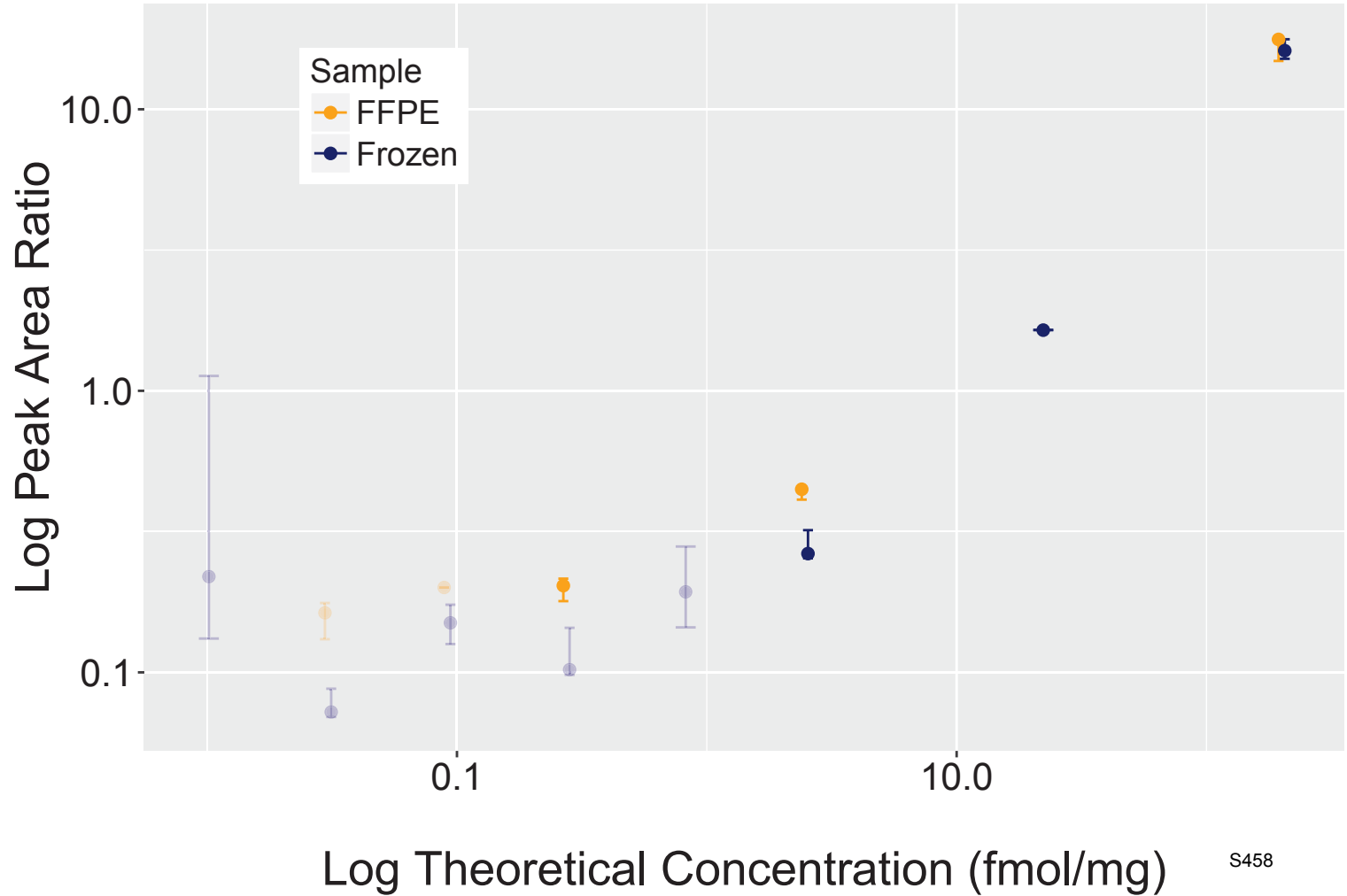
Analyte: ERBB2.NPQLC[+57]YQDTILWK



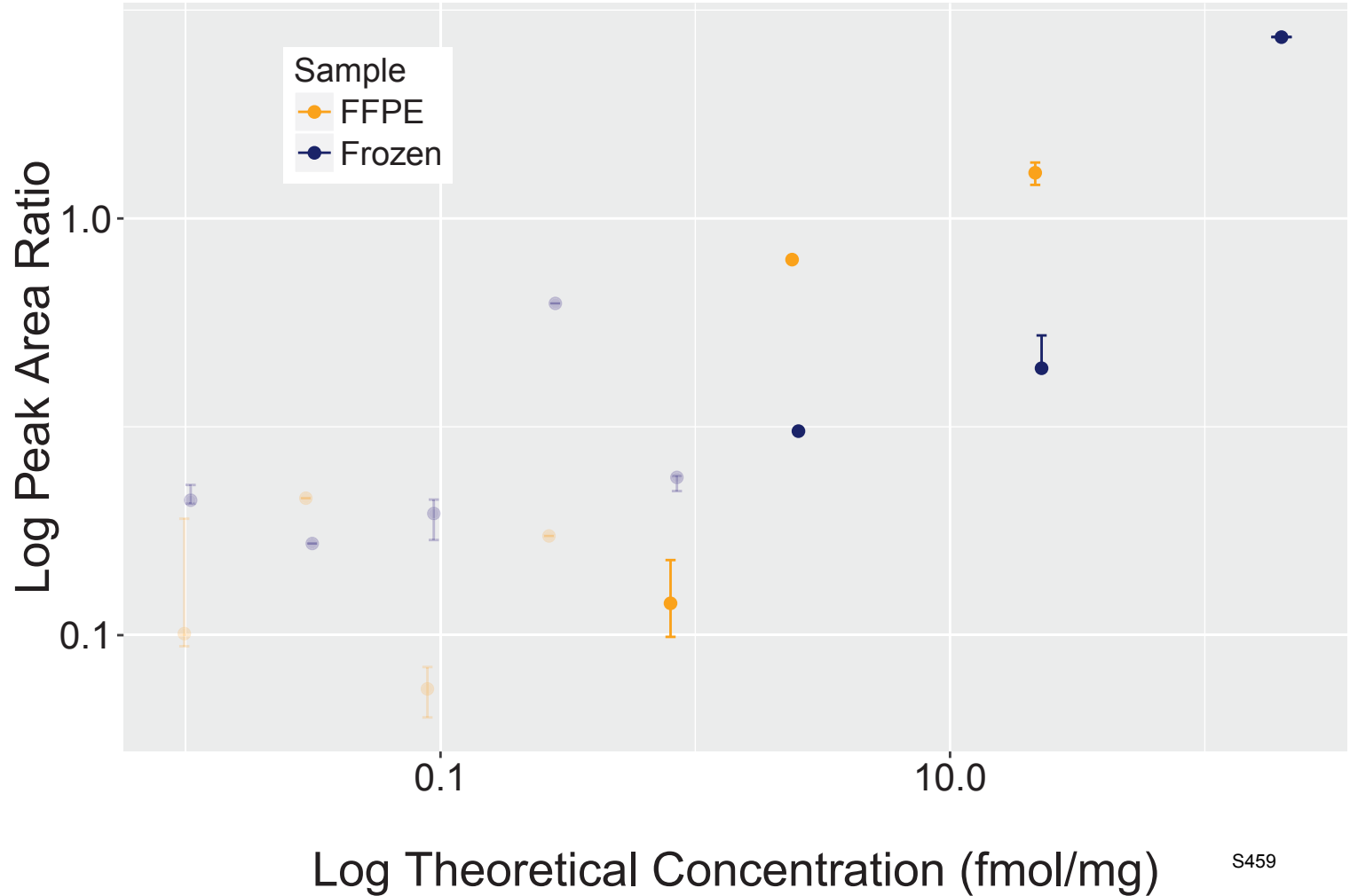
Analyte: GGCT.NPSAAFFC[+57]VAR



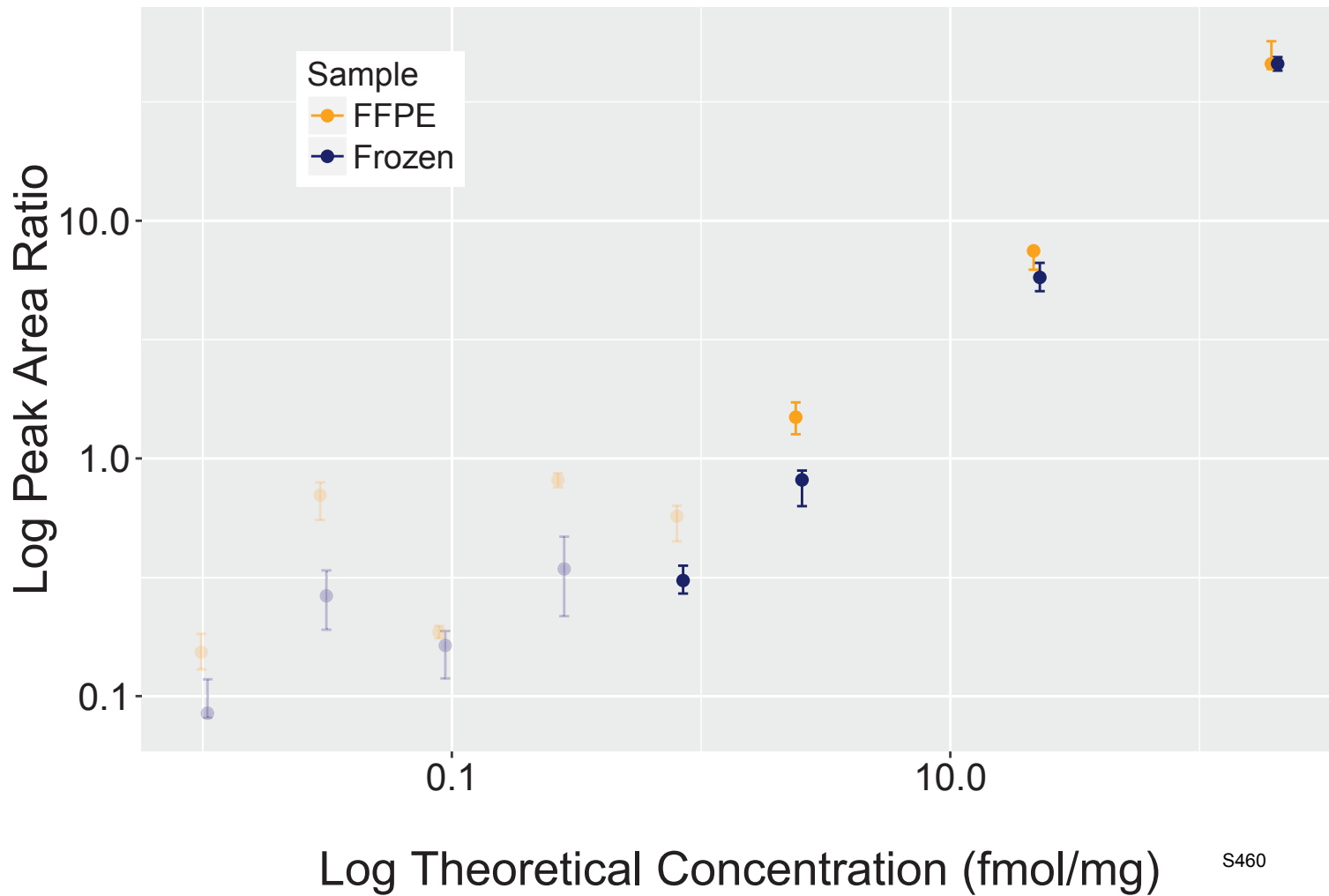
Analyte: CAPN2.NPWGEVEWTGR



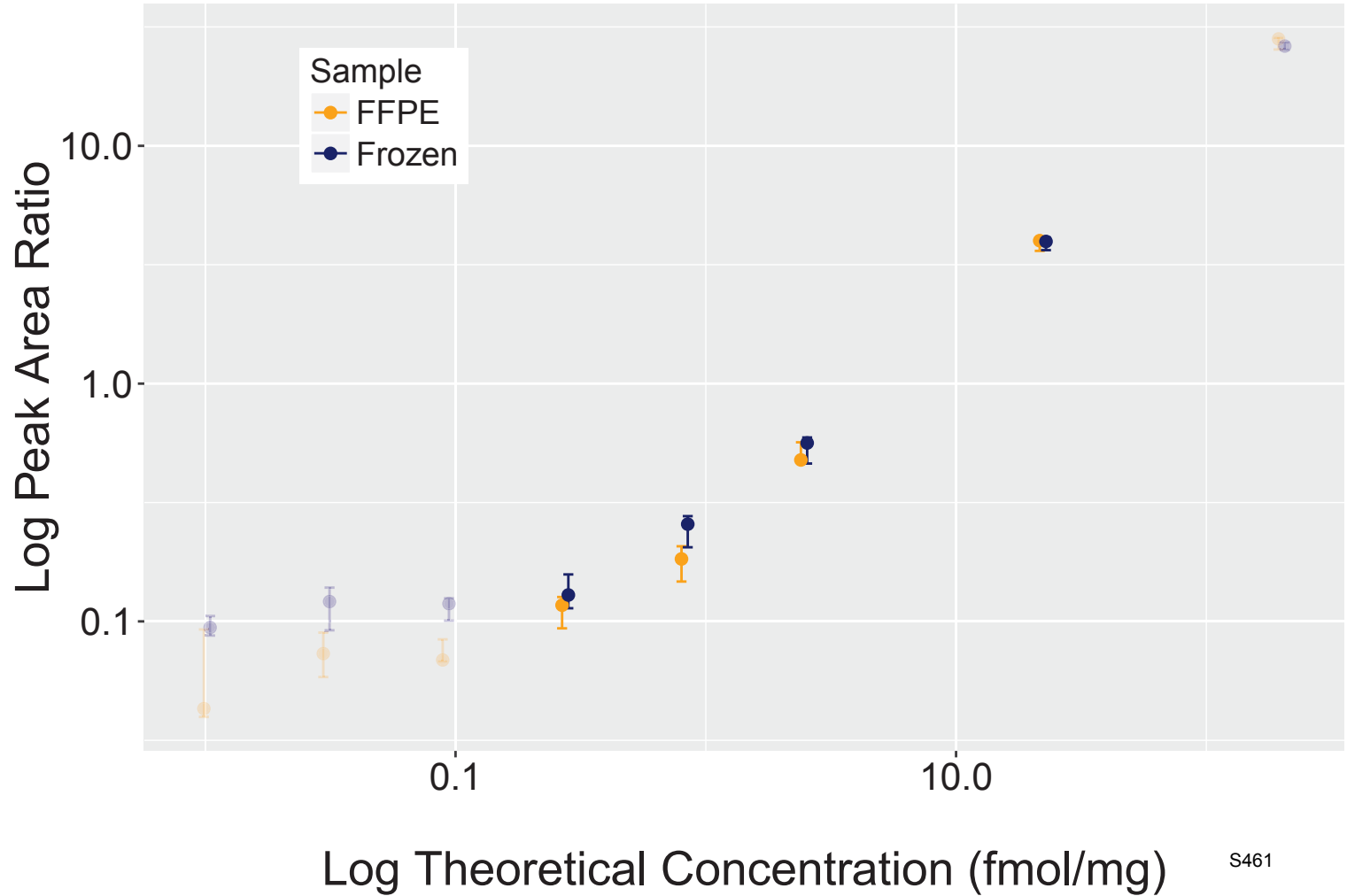
Analyte: PEBP1.NRPTSISWDGLDSGK



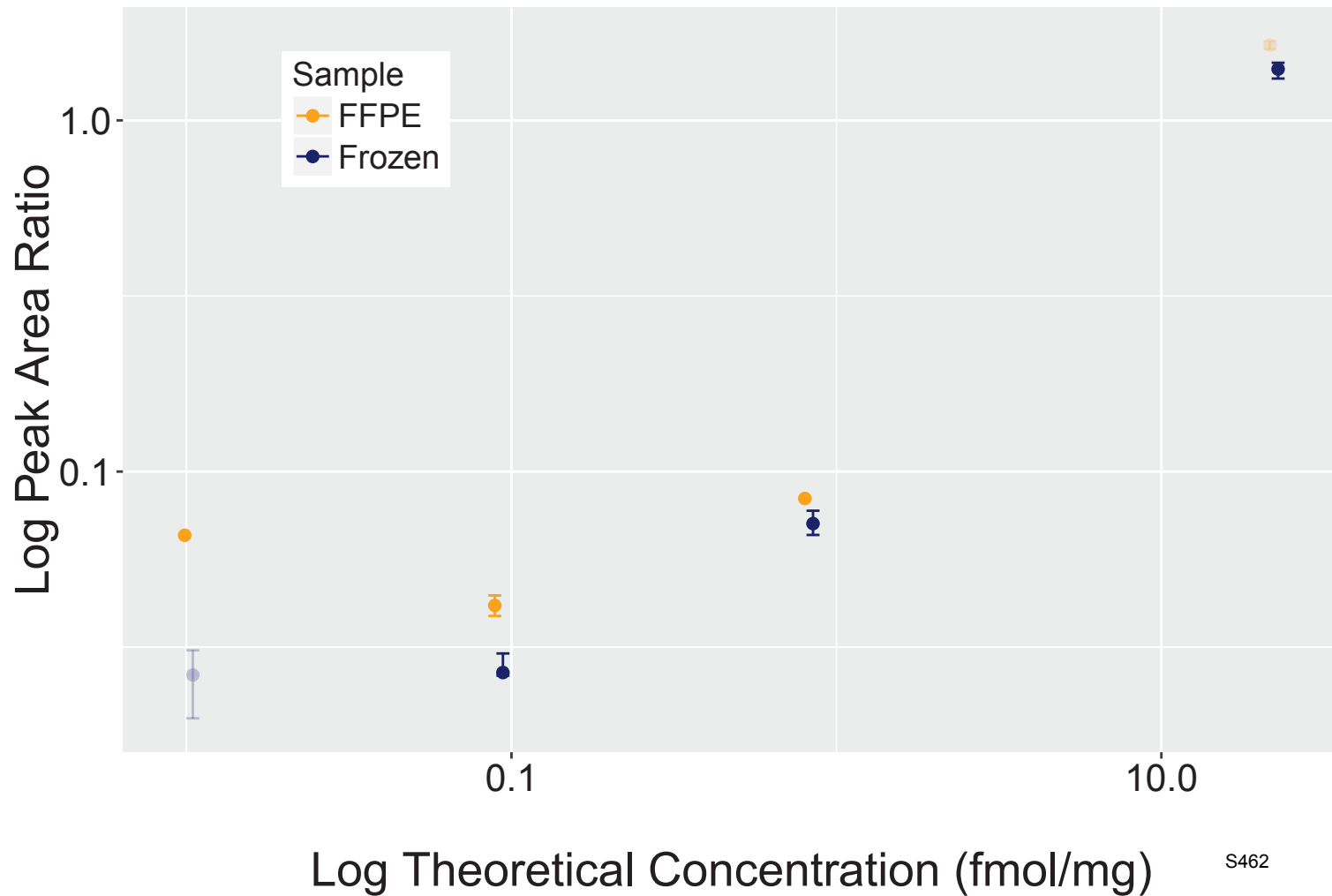
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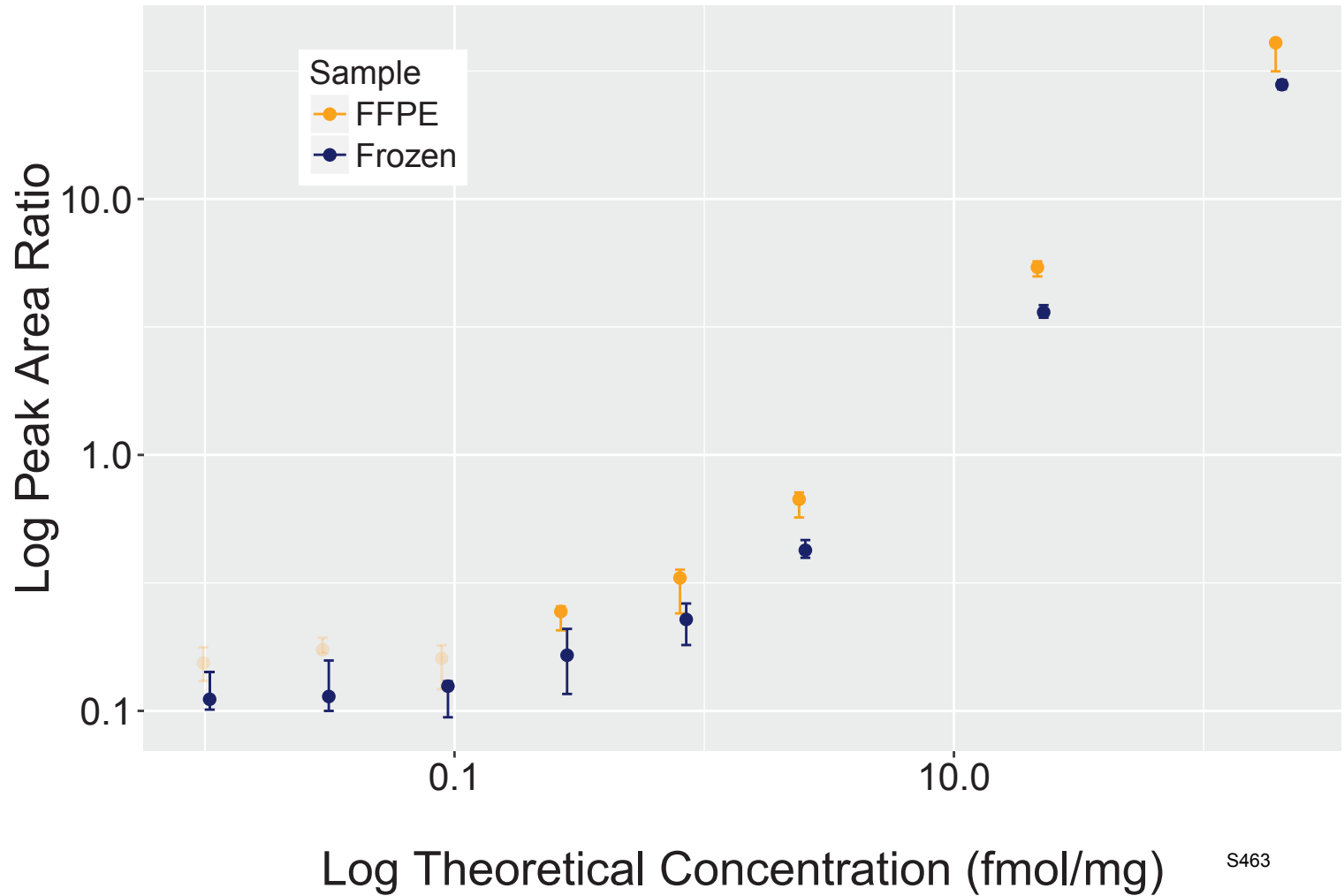
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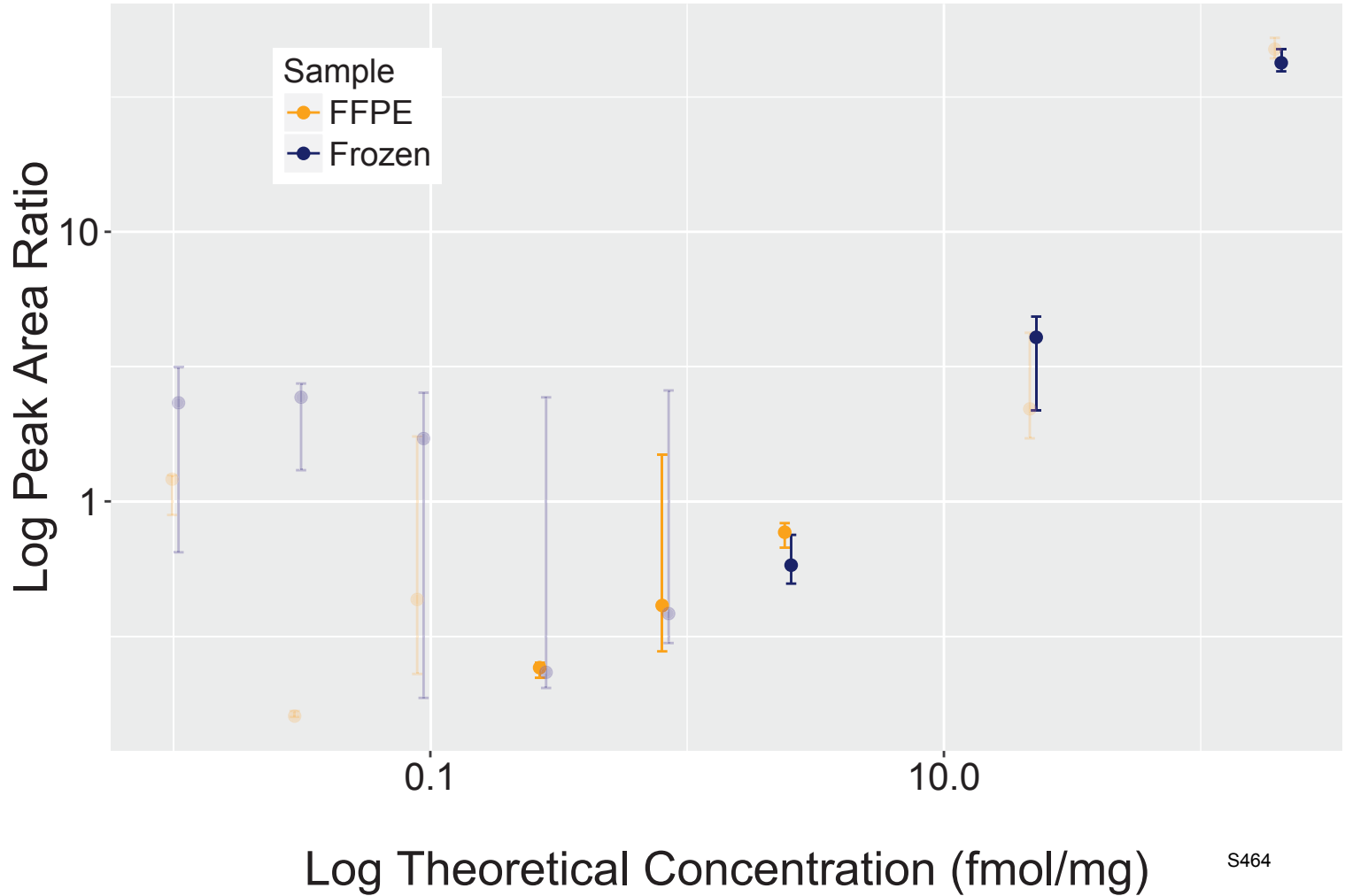
Analyte: CTSZ.NSWGEPWGER



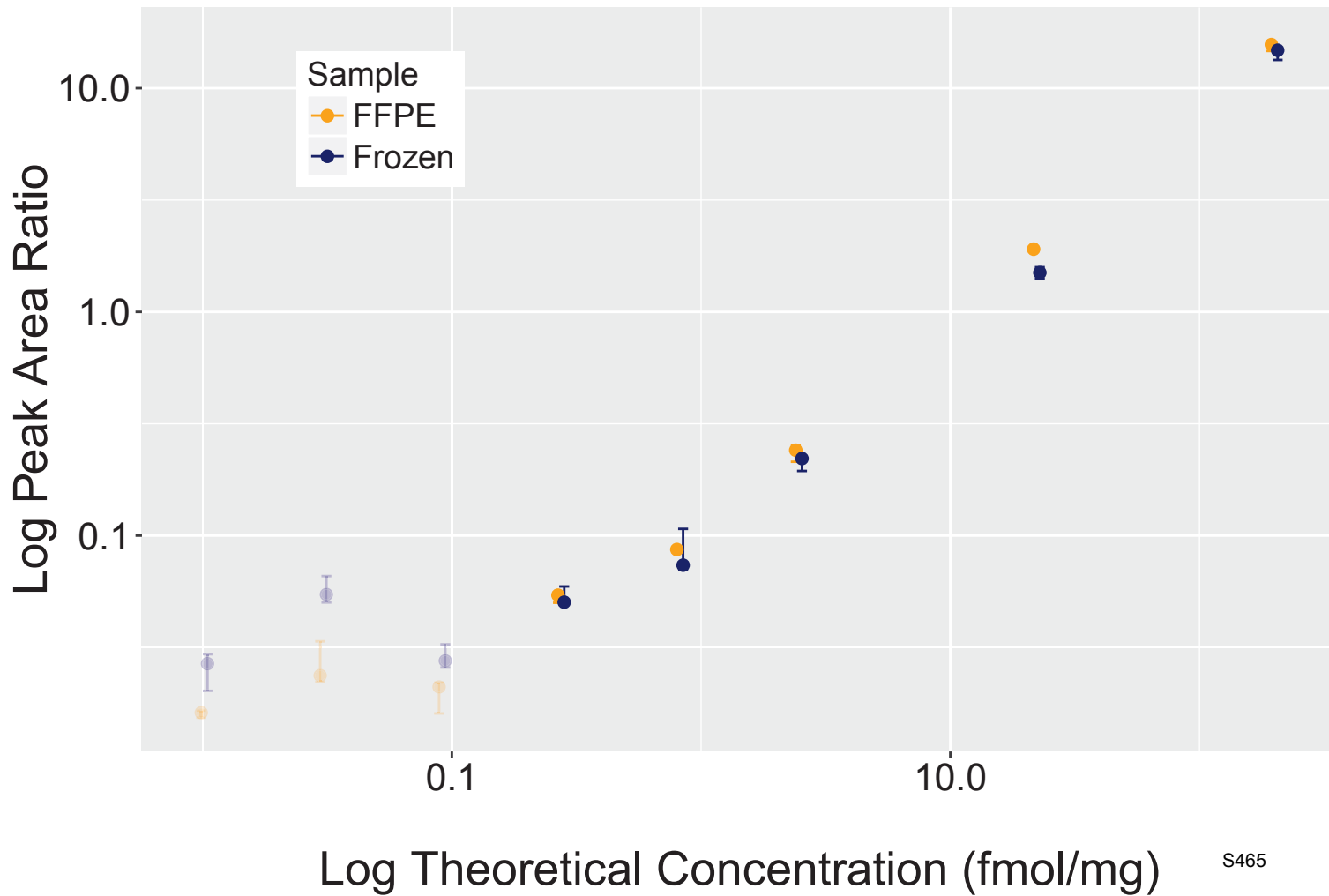
Analyte: G6PD.NSYVAGQYDDAASYQR



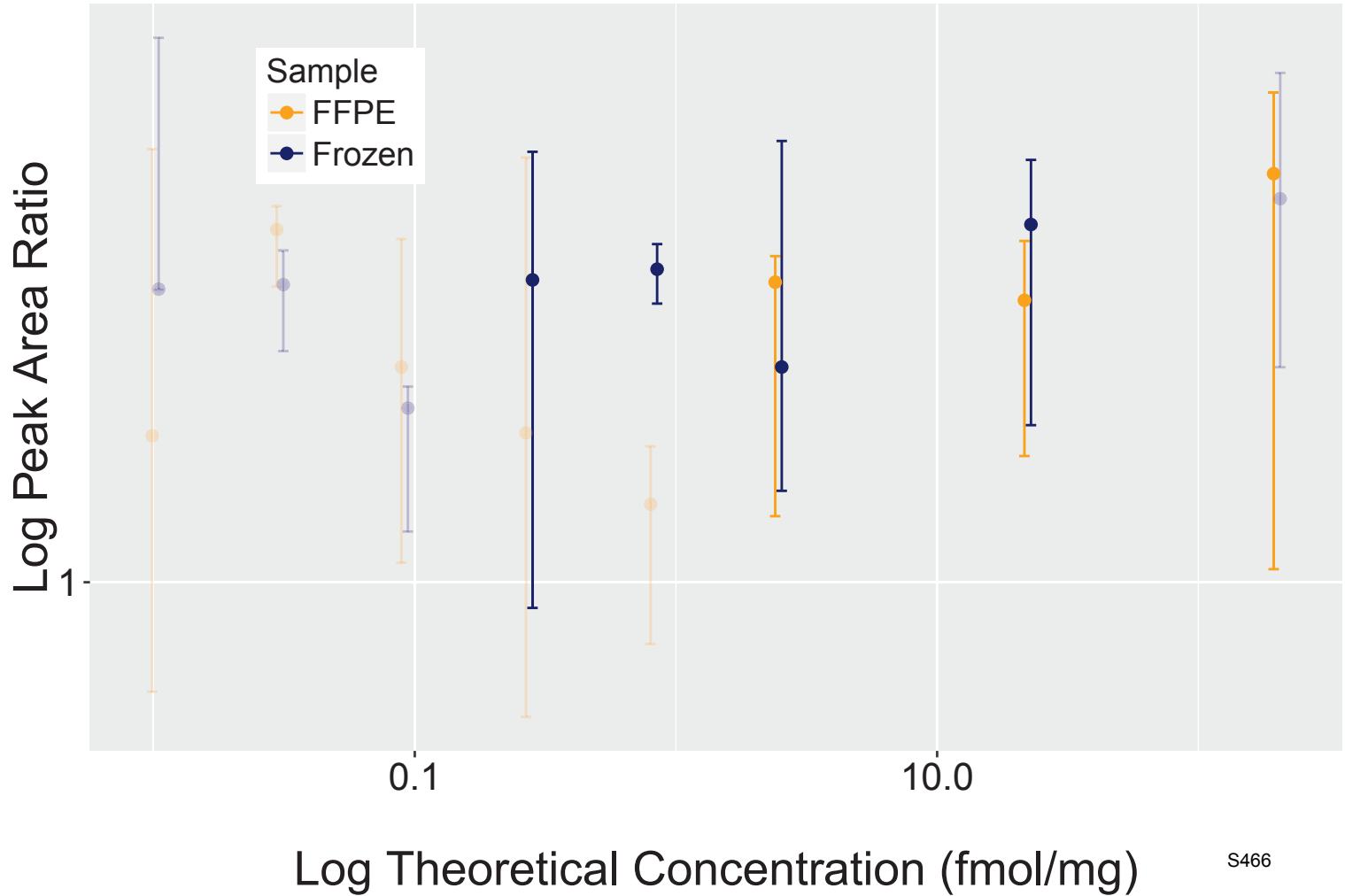
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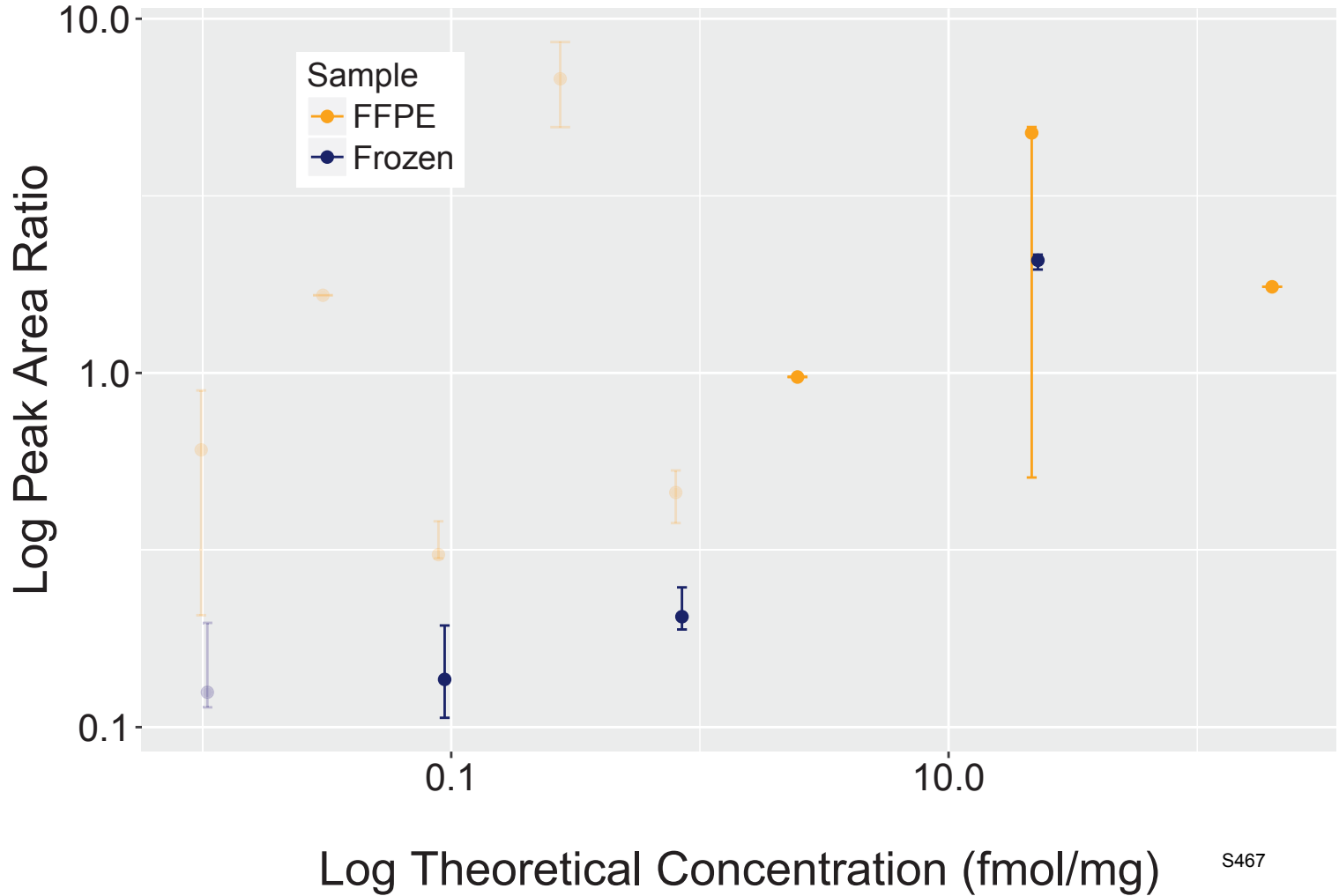
Analyte: CTSZ.NVDGVNYASITR



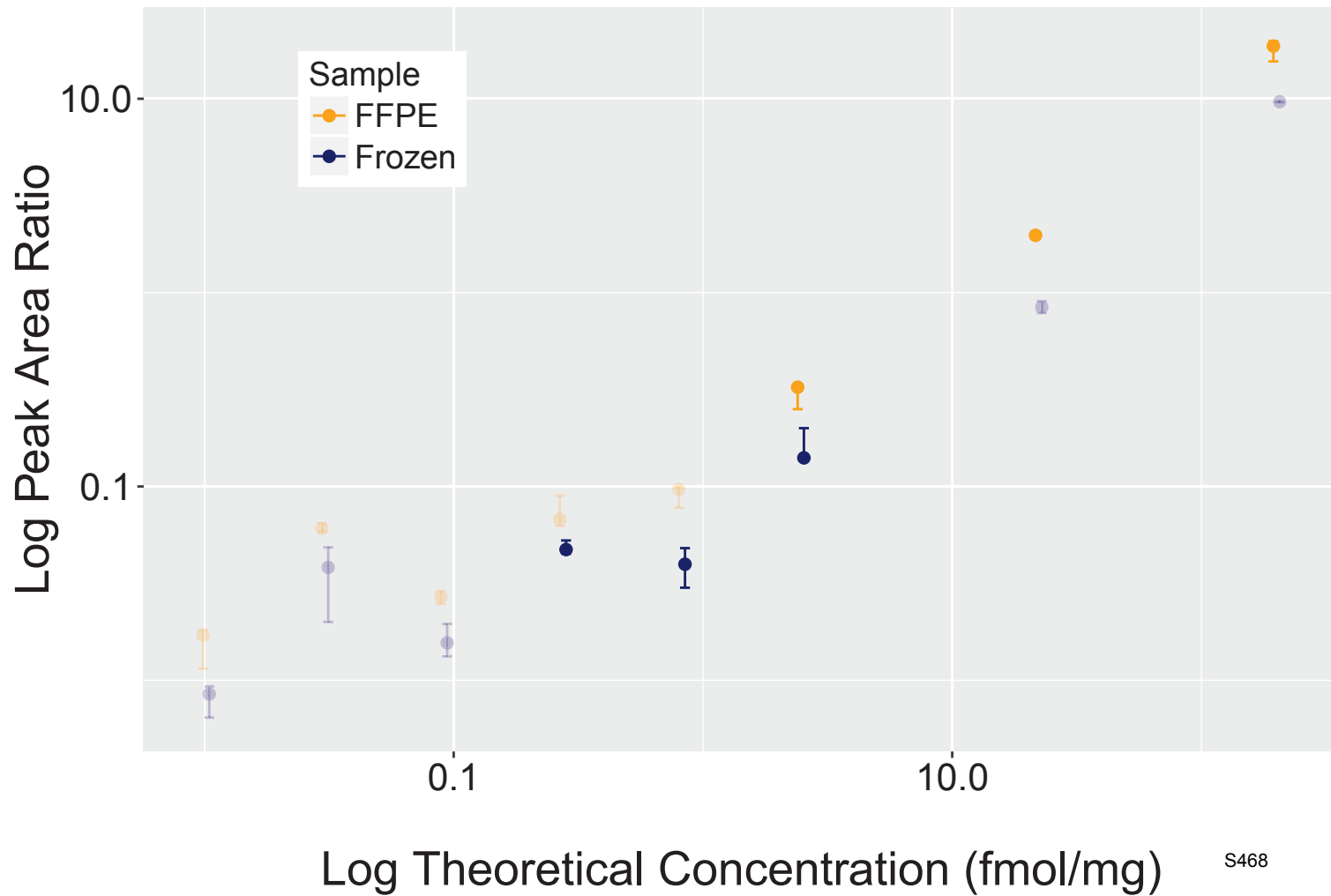
Analyte: HPRT1.NVLIVEDIIDTGK



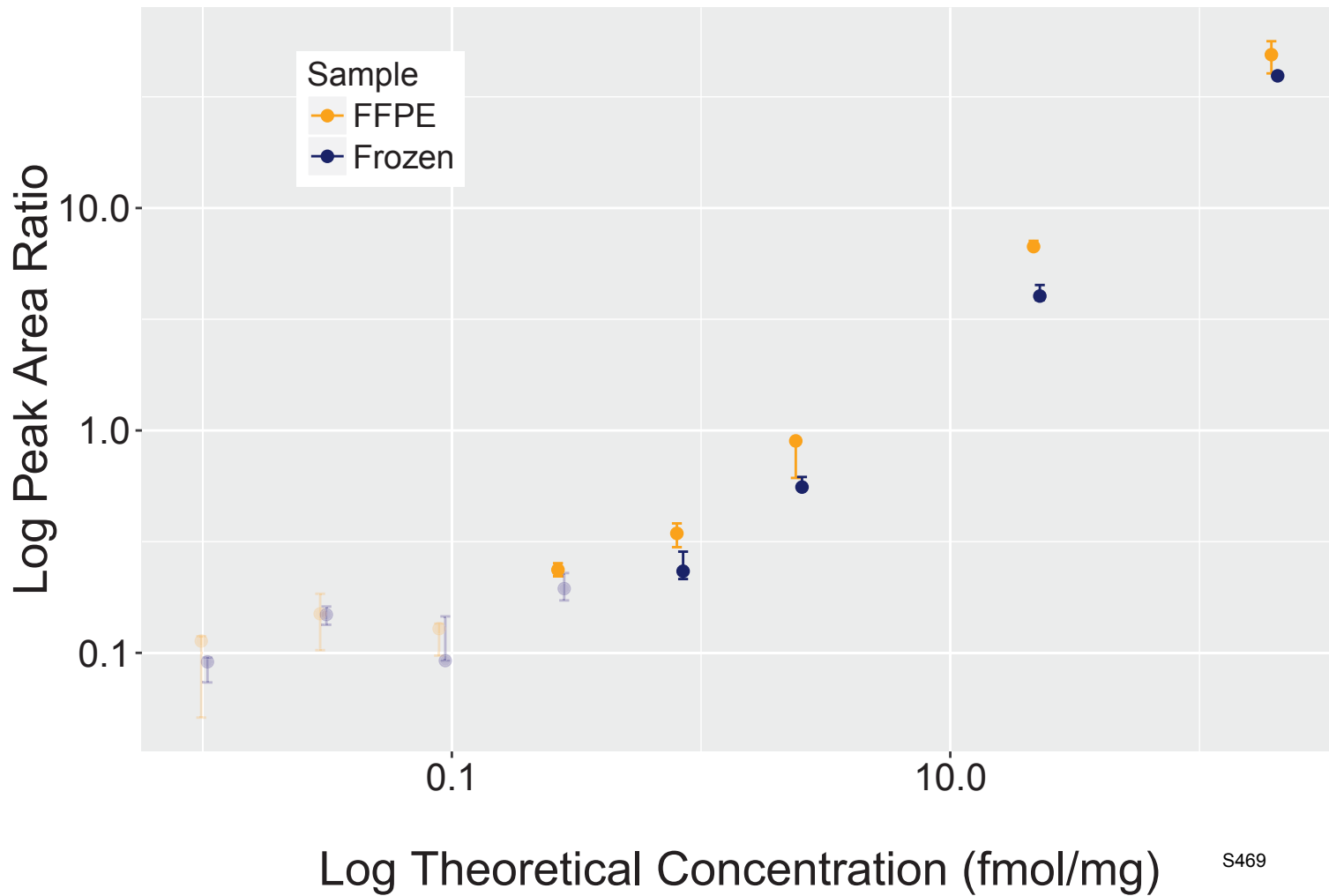
Analyte: ACTN4.NVNVQNFHISWK



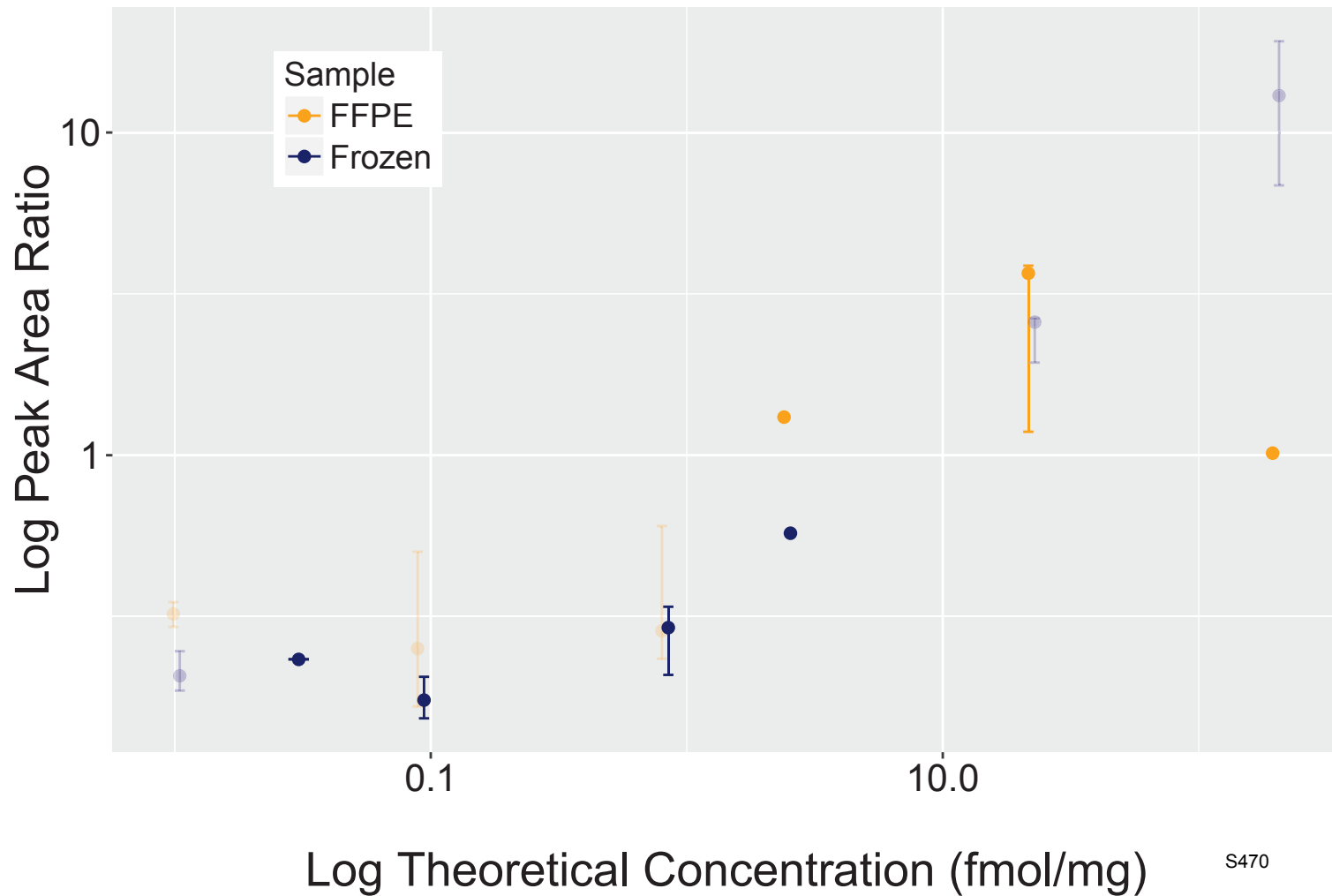
Analyte: YWHAG.NVTELNEPLSNEER



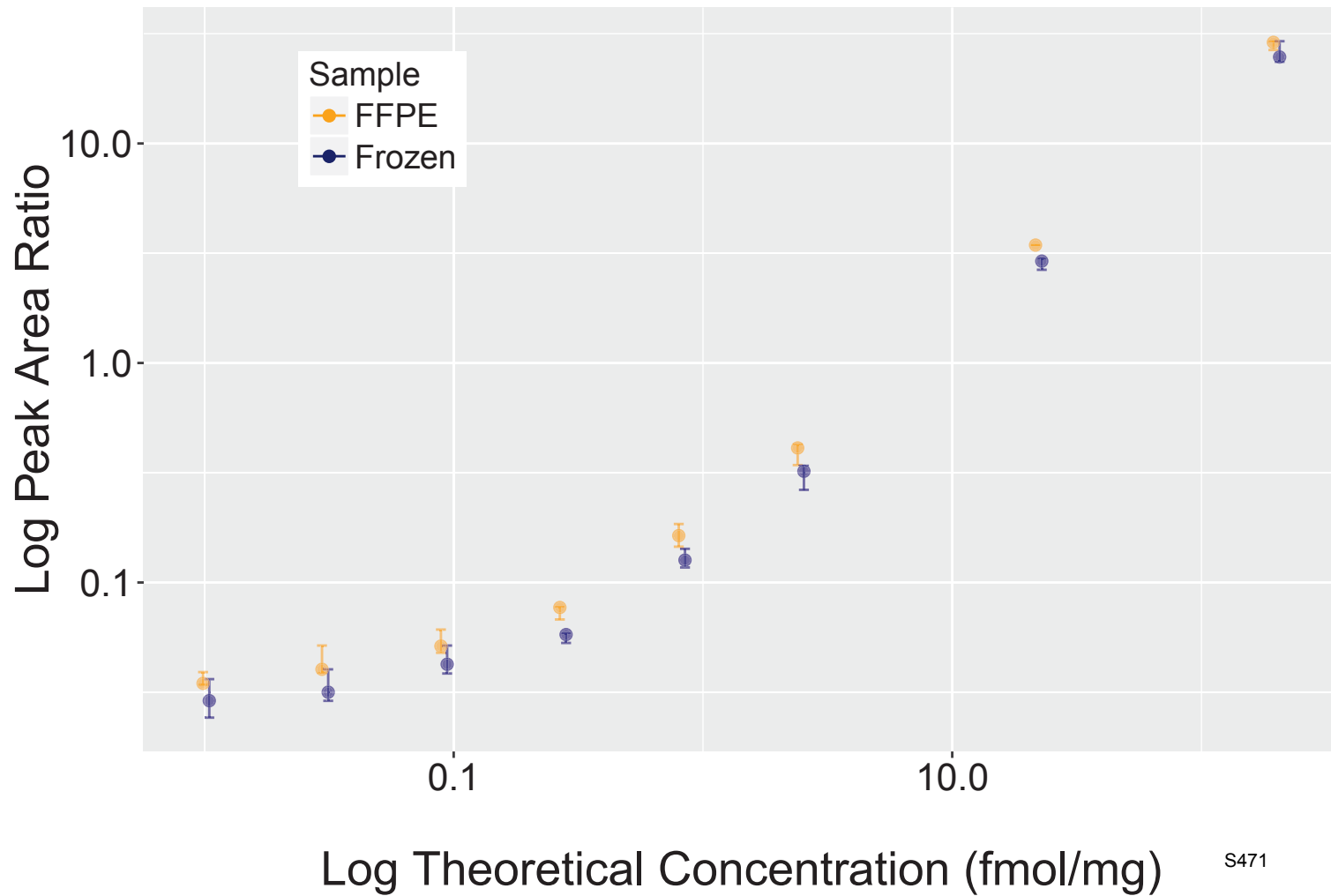
Analyte: MUC1.NYGQLDIFPAR



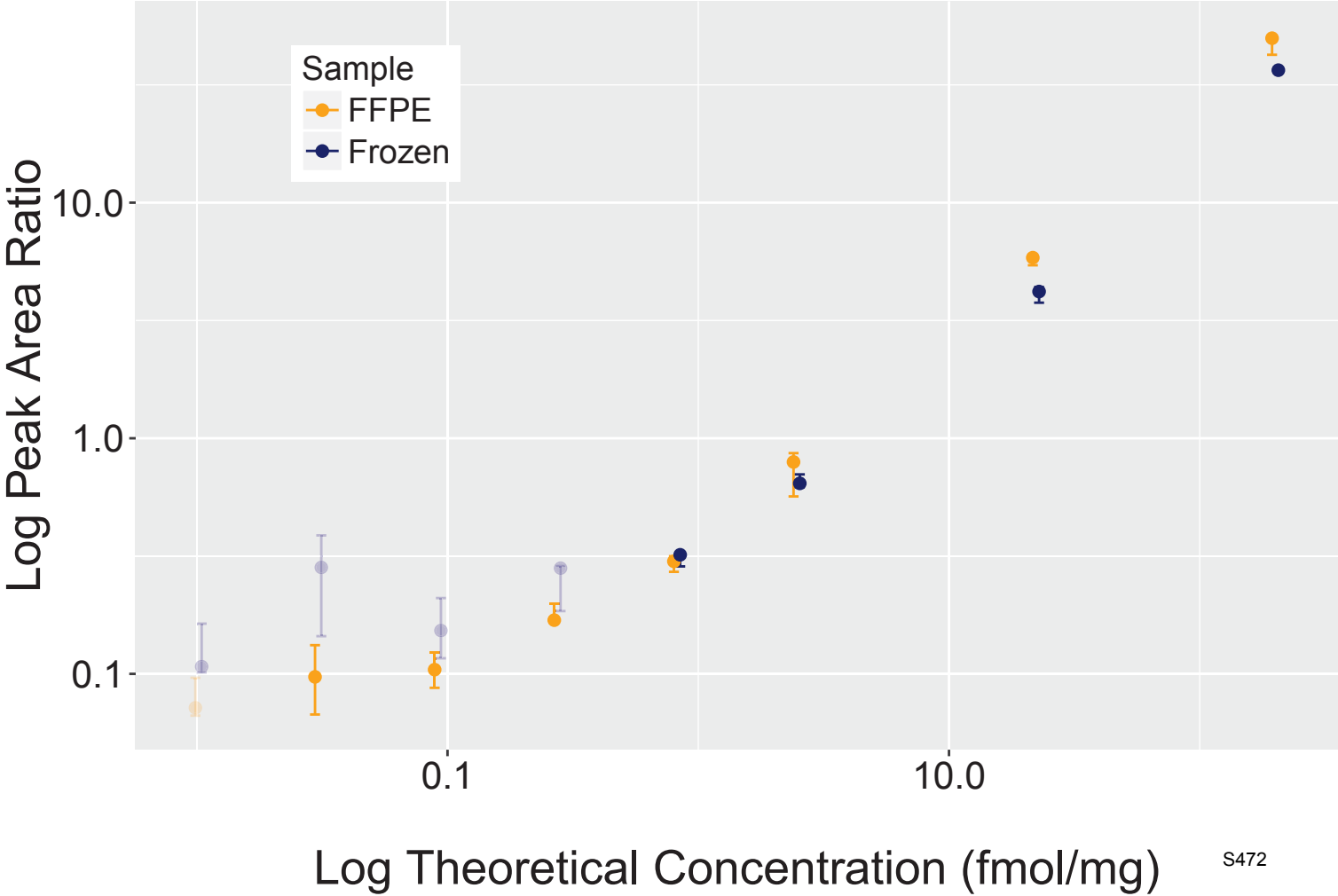
Analyte: CAPN1.NYPATFWVNPQFK



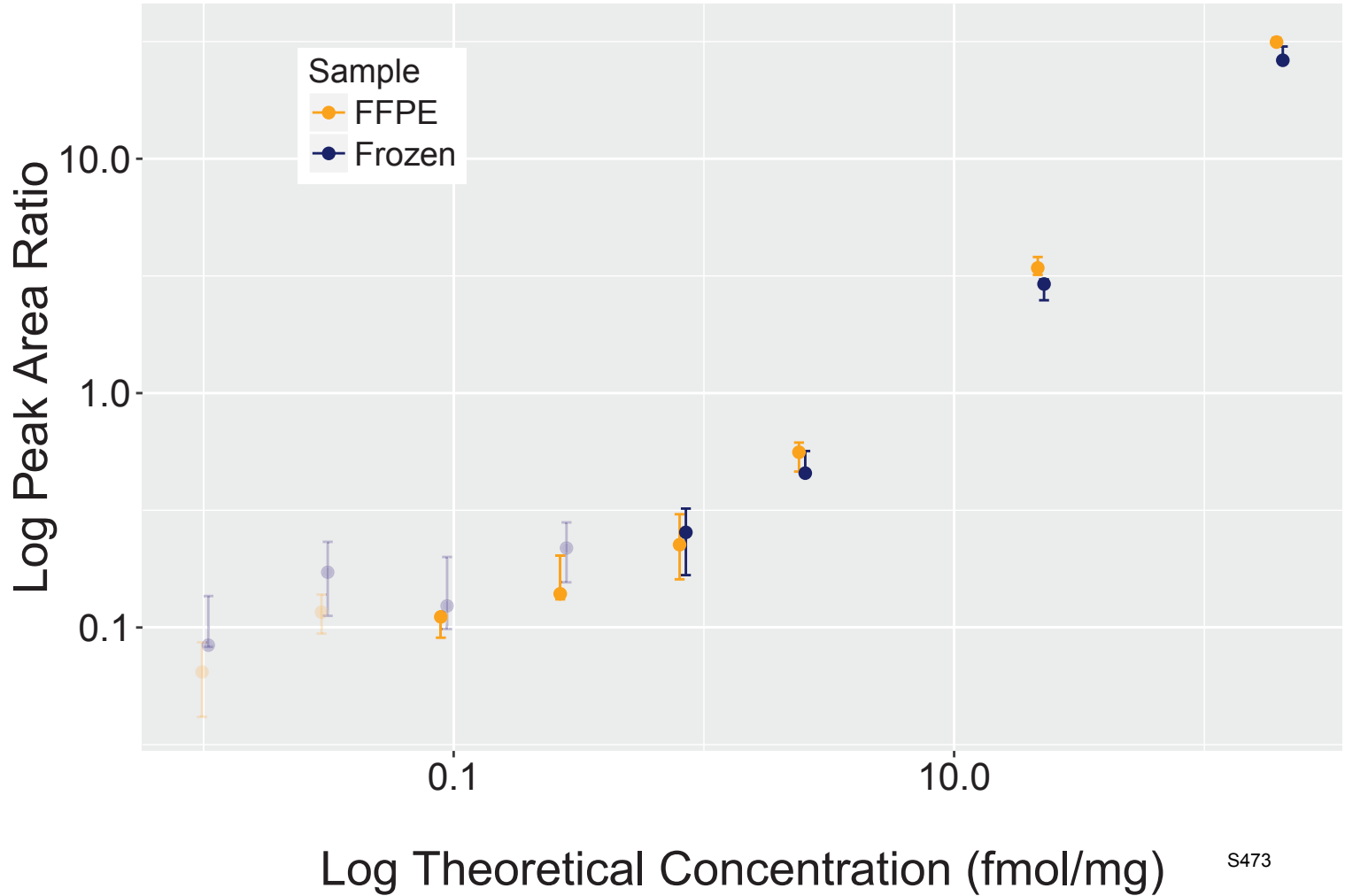
Analyte: USP5.SAADSISESVPVGPK



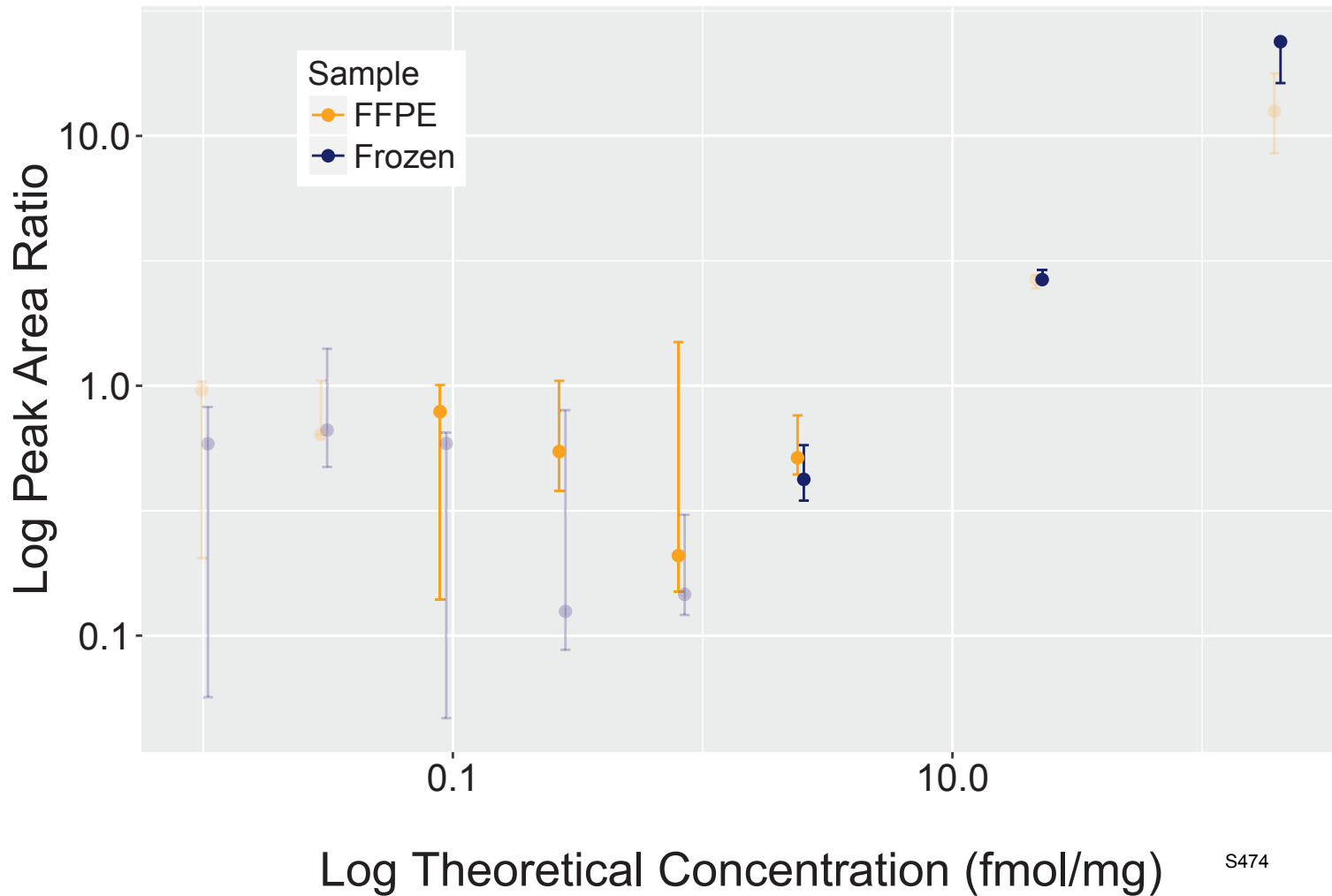
Analyte: CA2.SADFTNFDPR



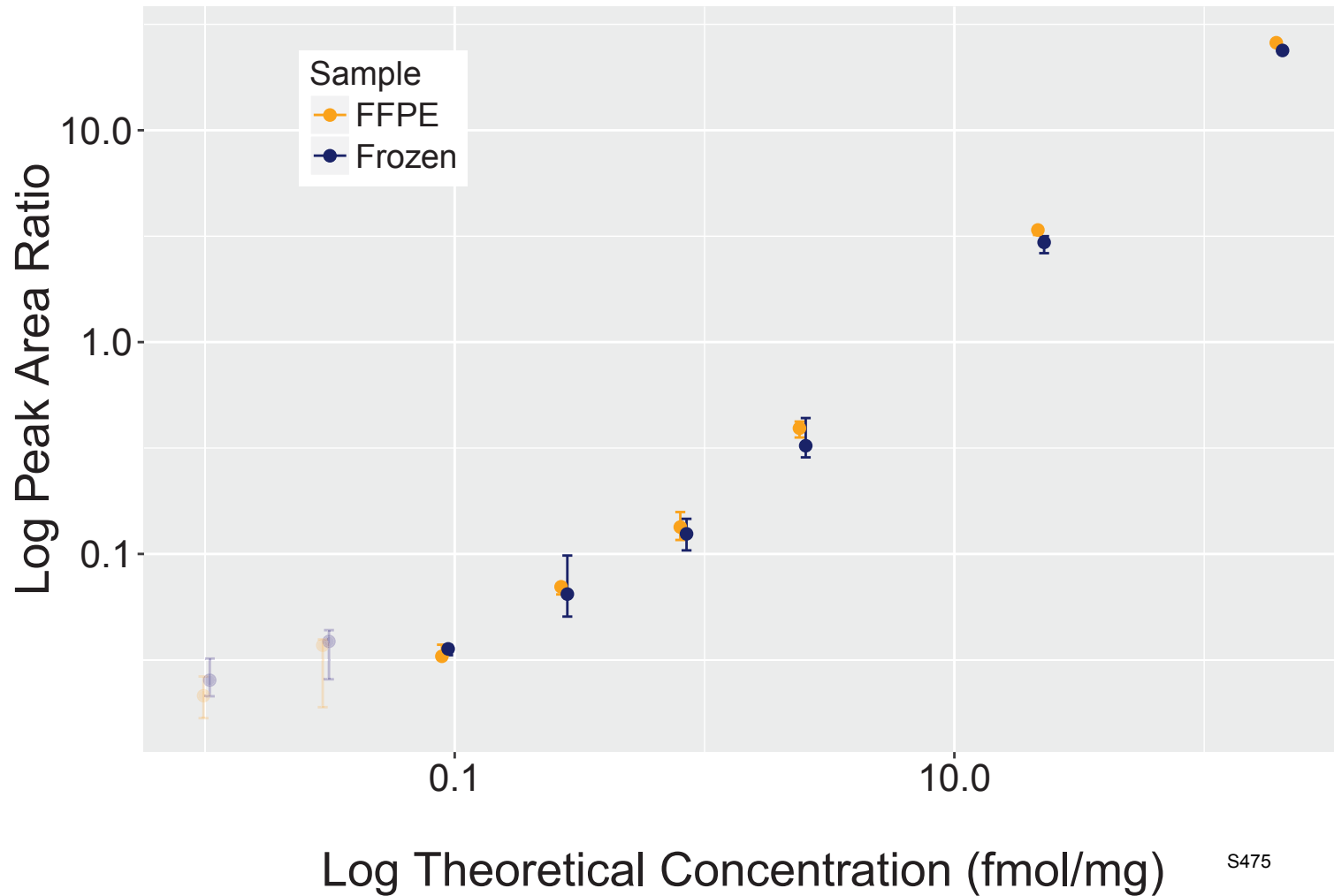
Analyte: KIF5B.SATLASIDAELQK



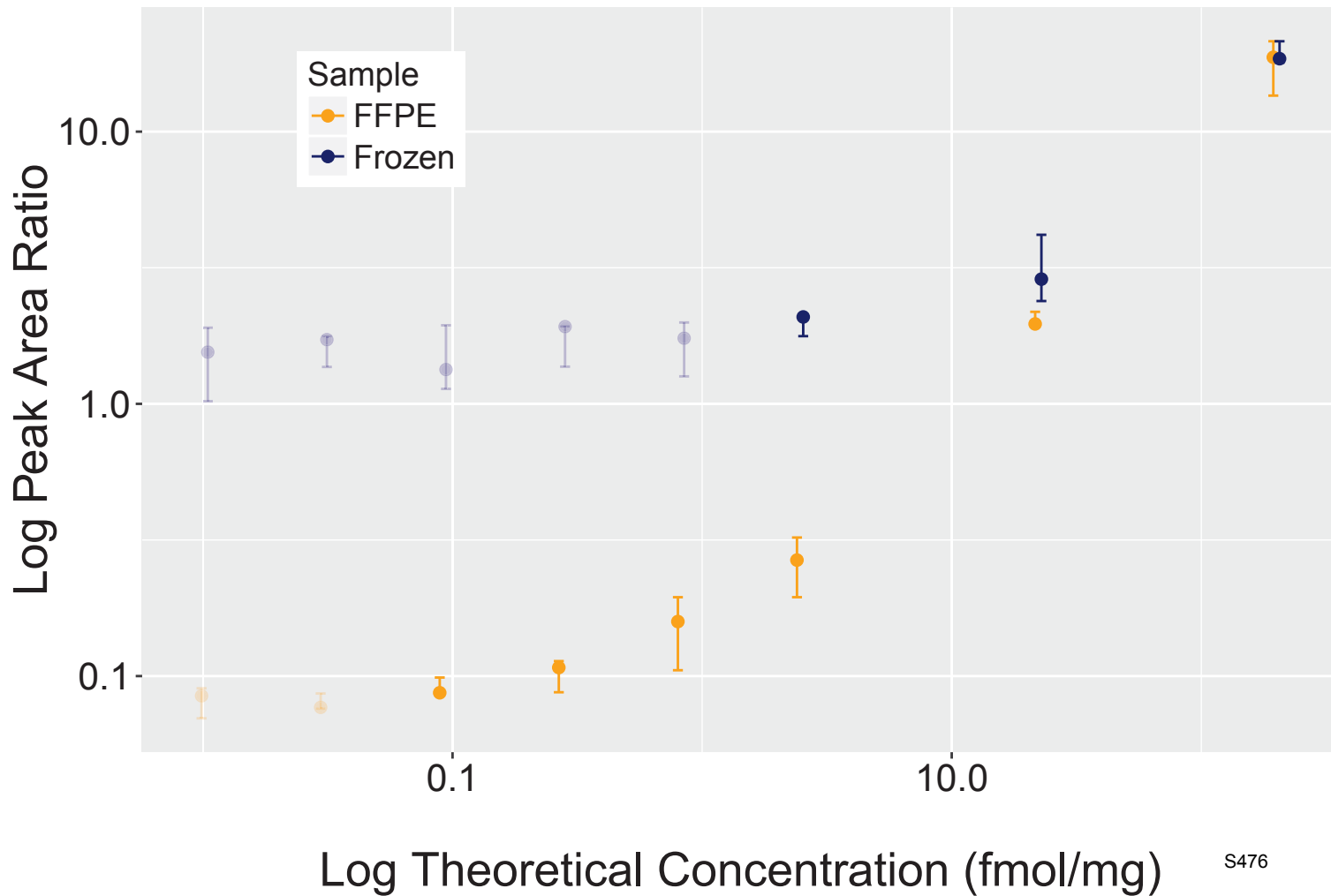
Analyte: XPO5.SC[+57]DPGLEDPG[+57]GLNR



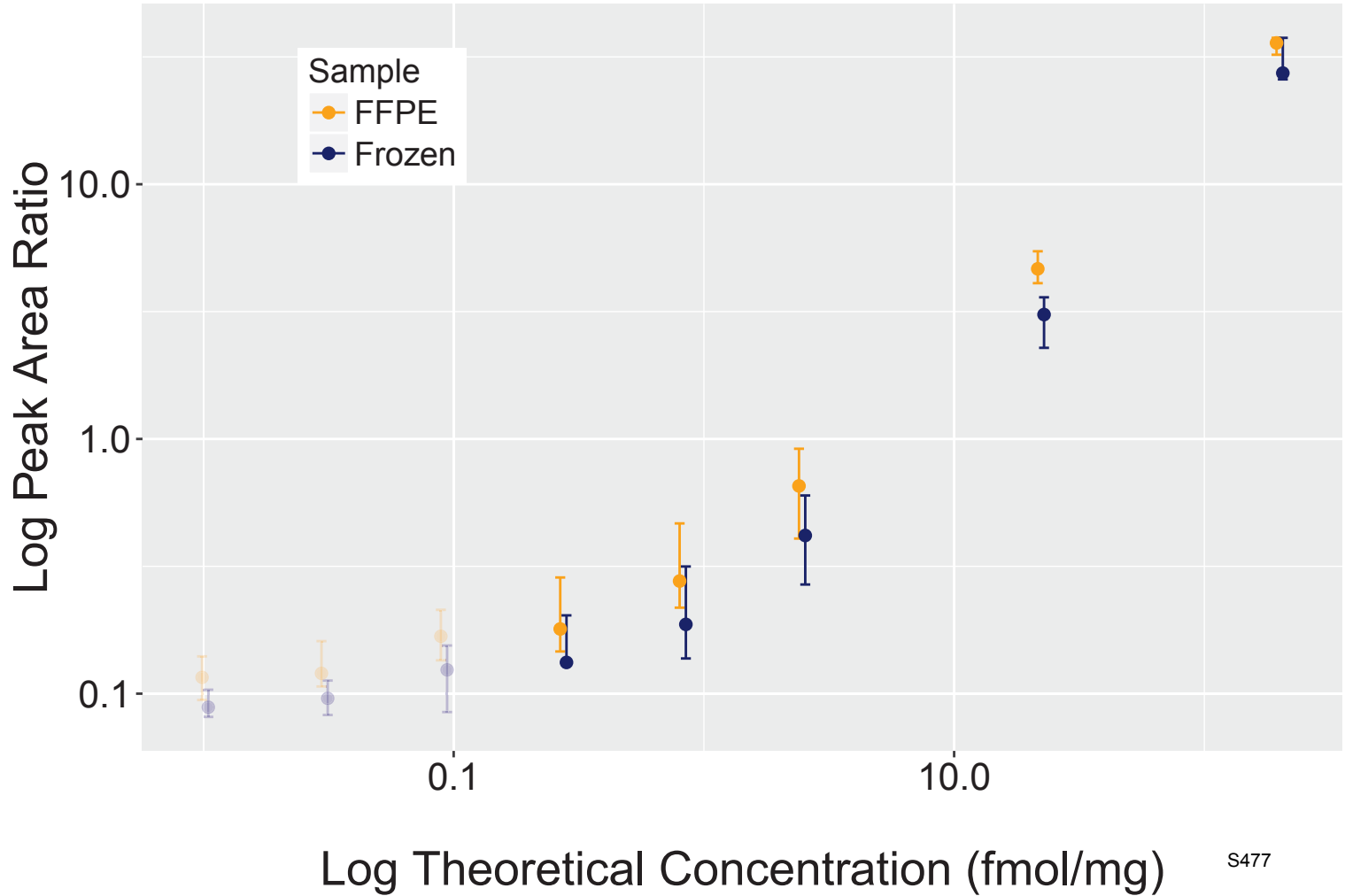
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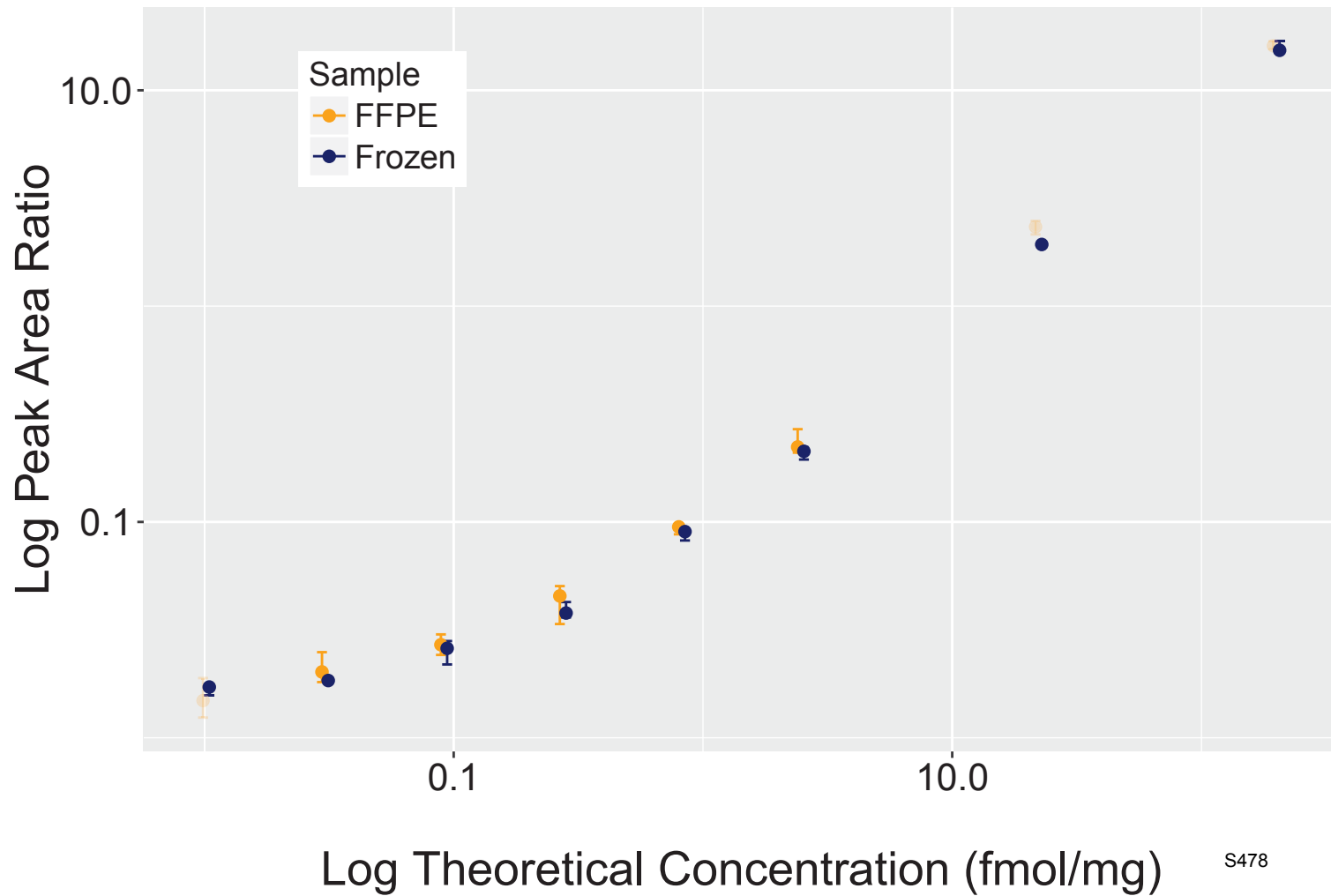
Analyte: LGALS3BP.SDLAVPSELALLK



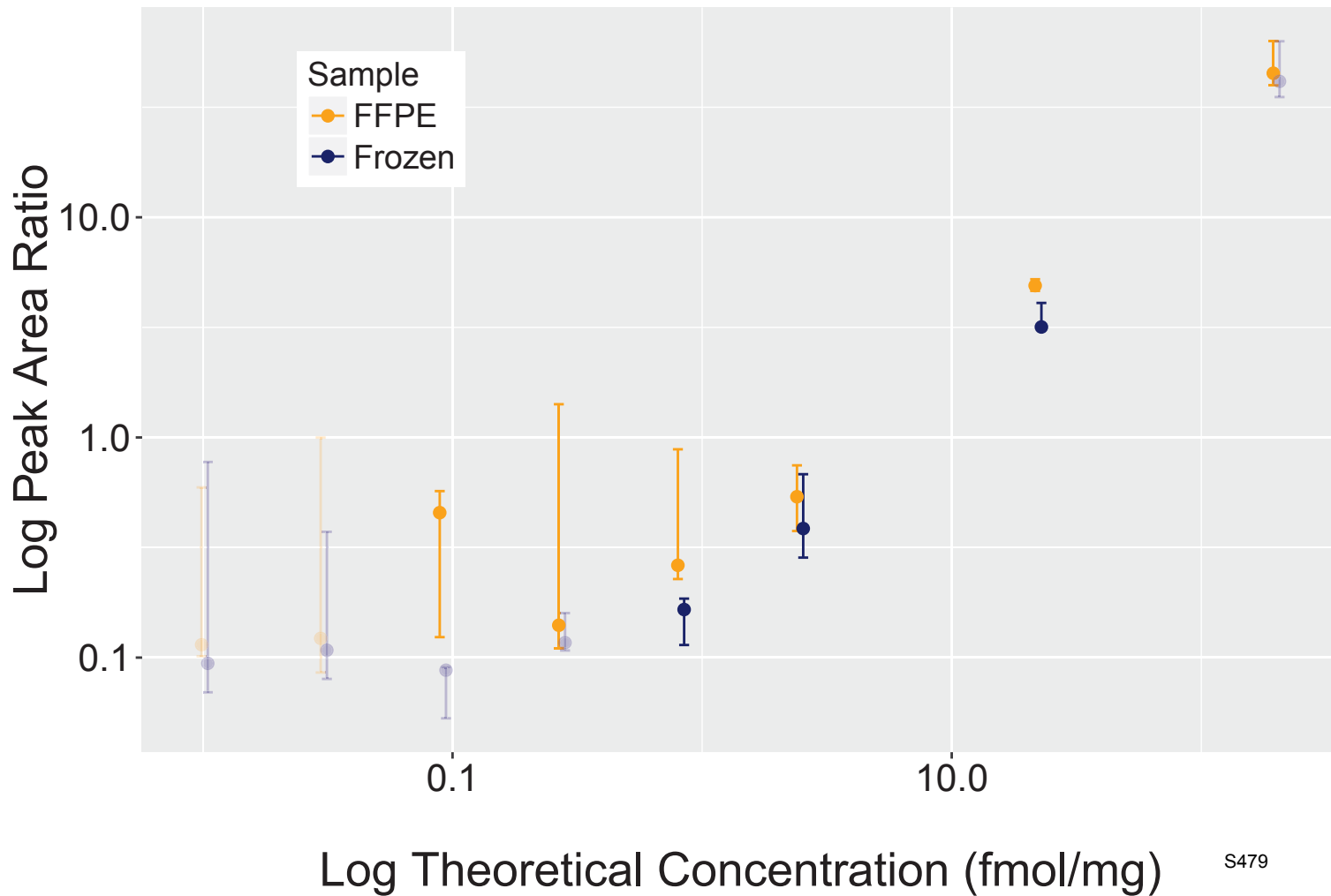
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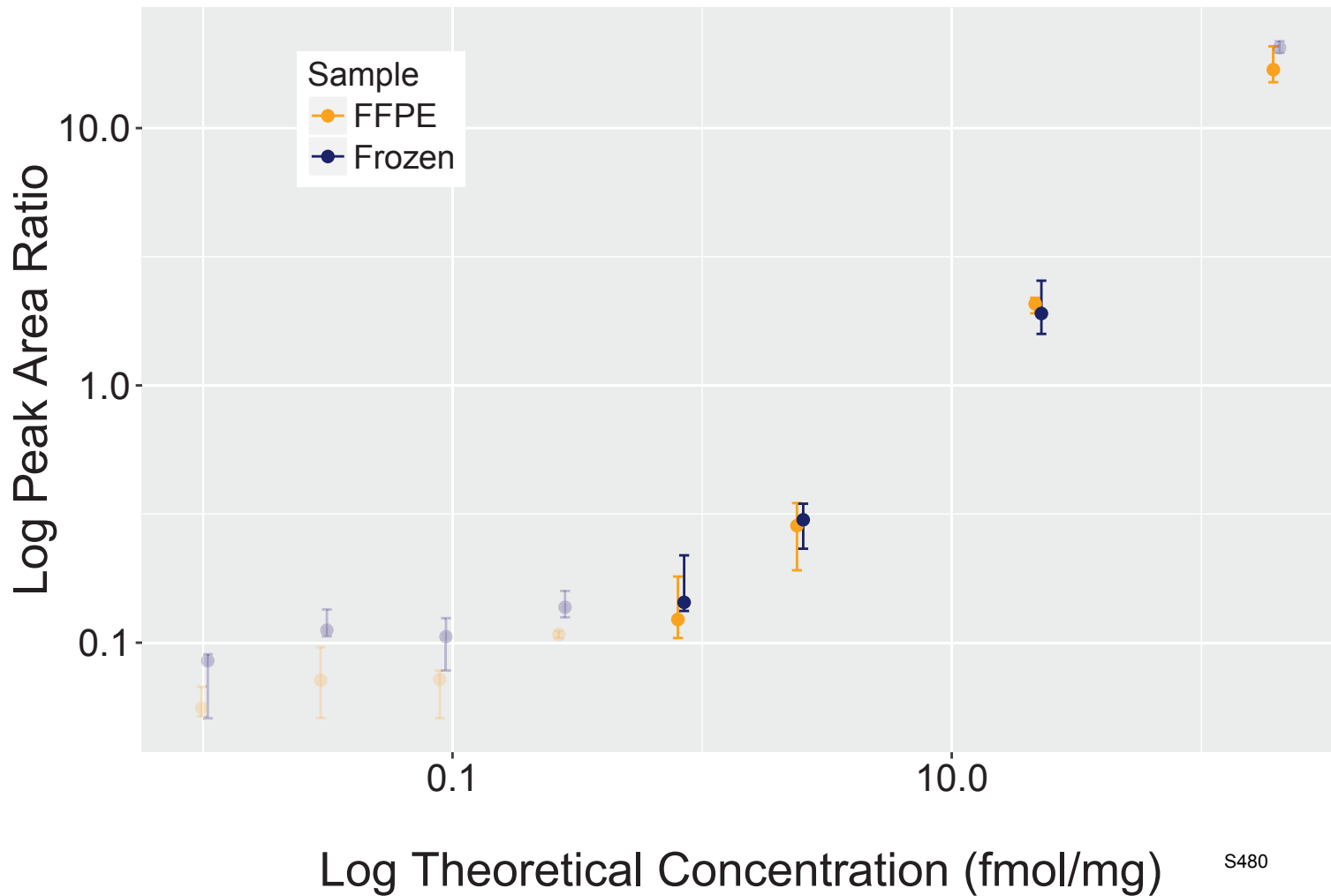
Analyte: CAPN2.SDTFINLR



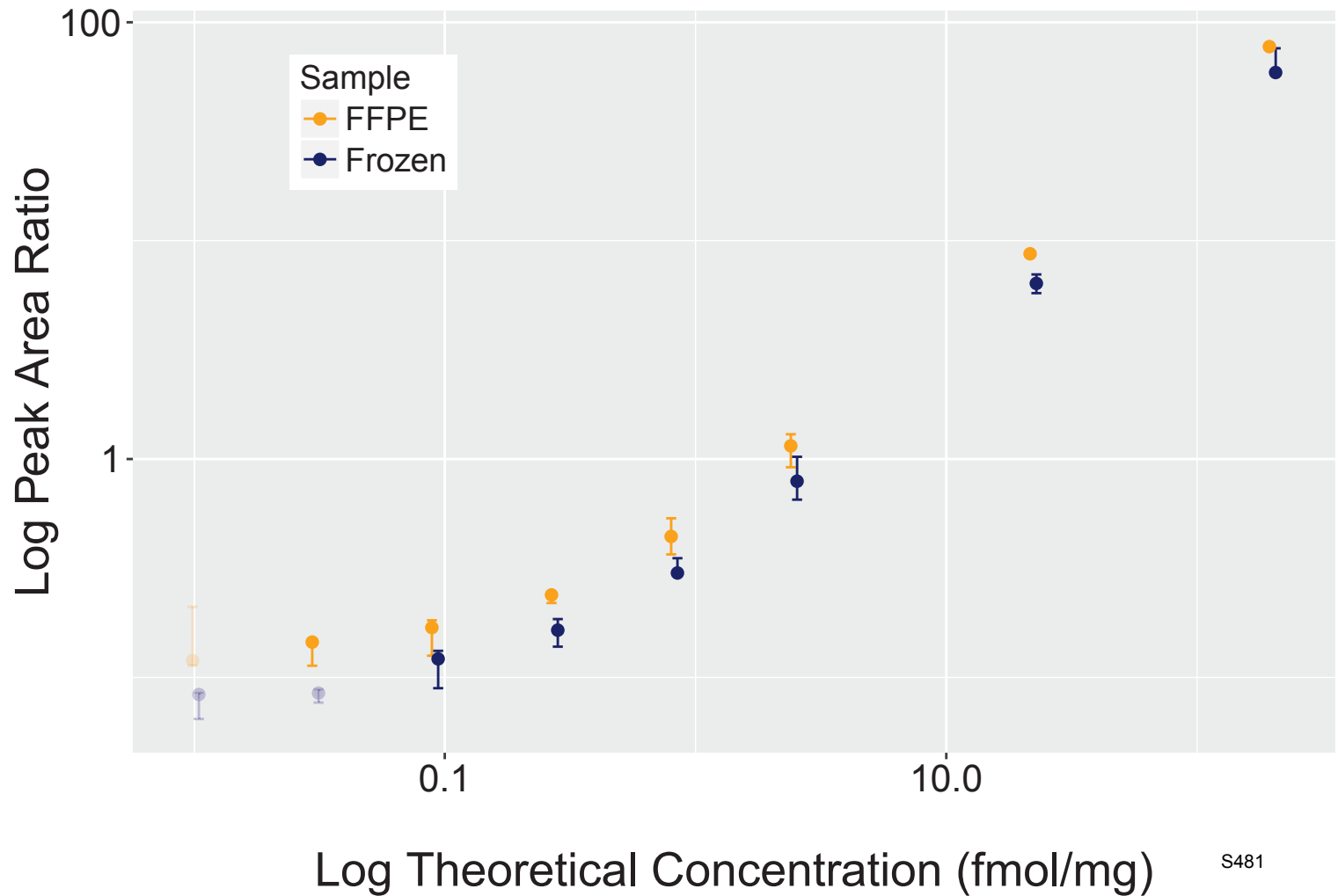
Analyte: PRKCSH.SEALPTDLPAPSAPDLTEPK



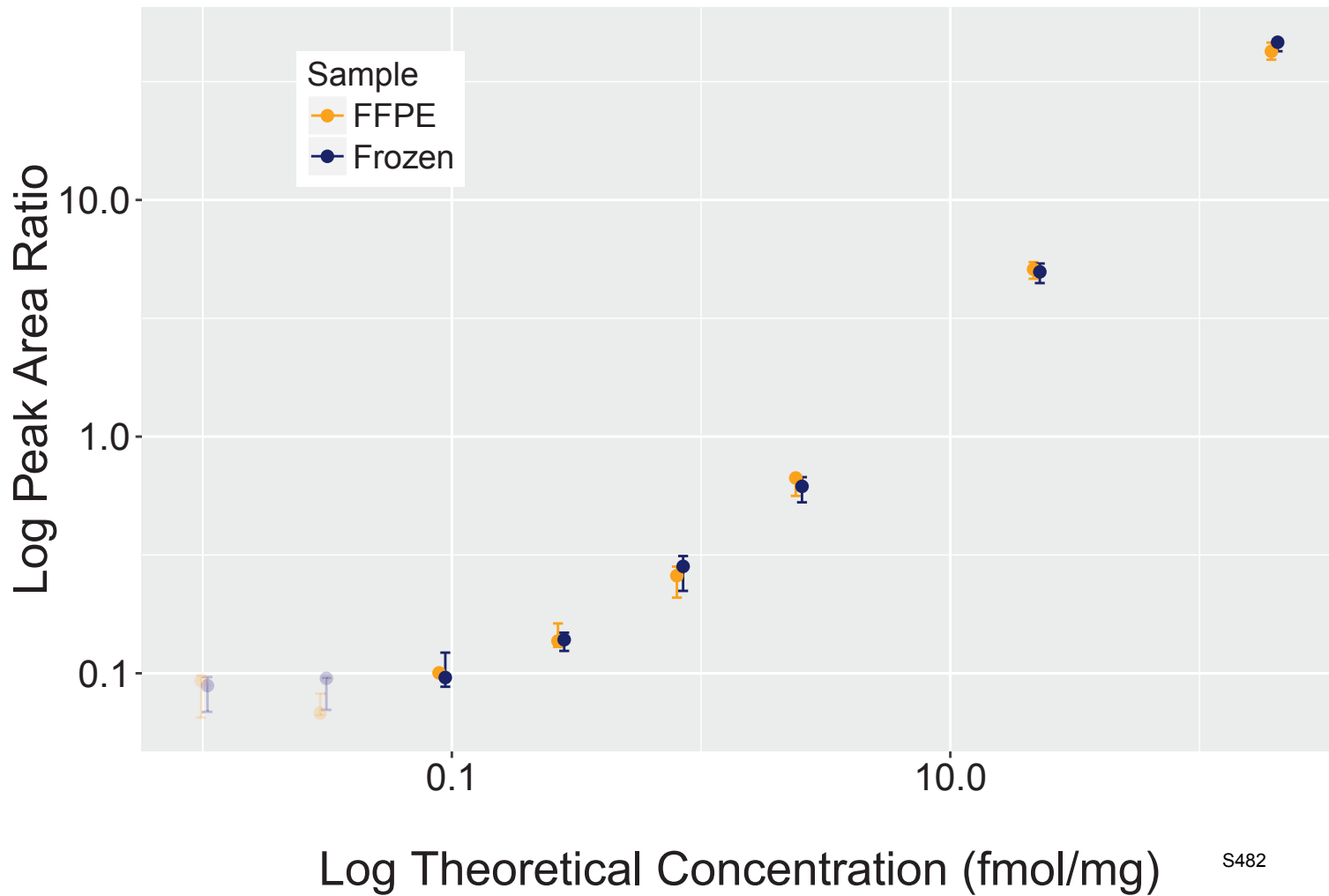
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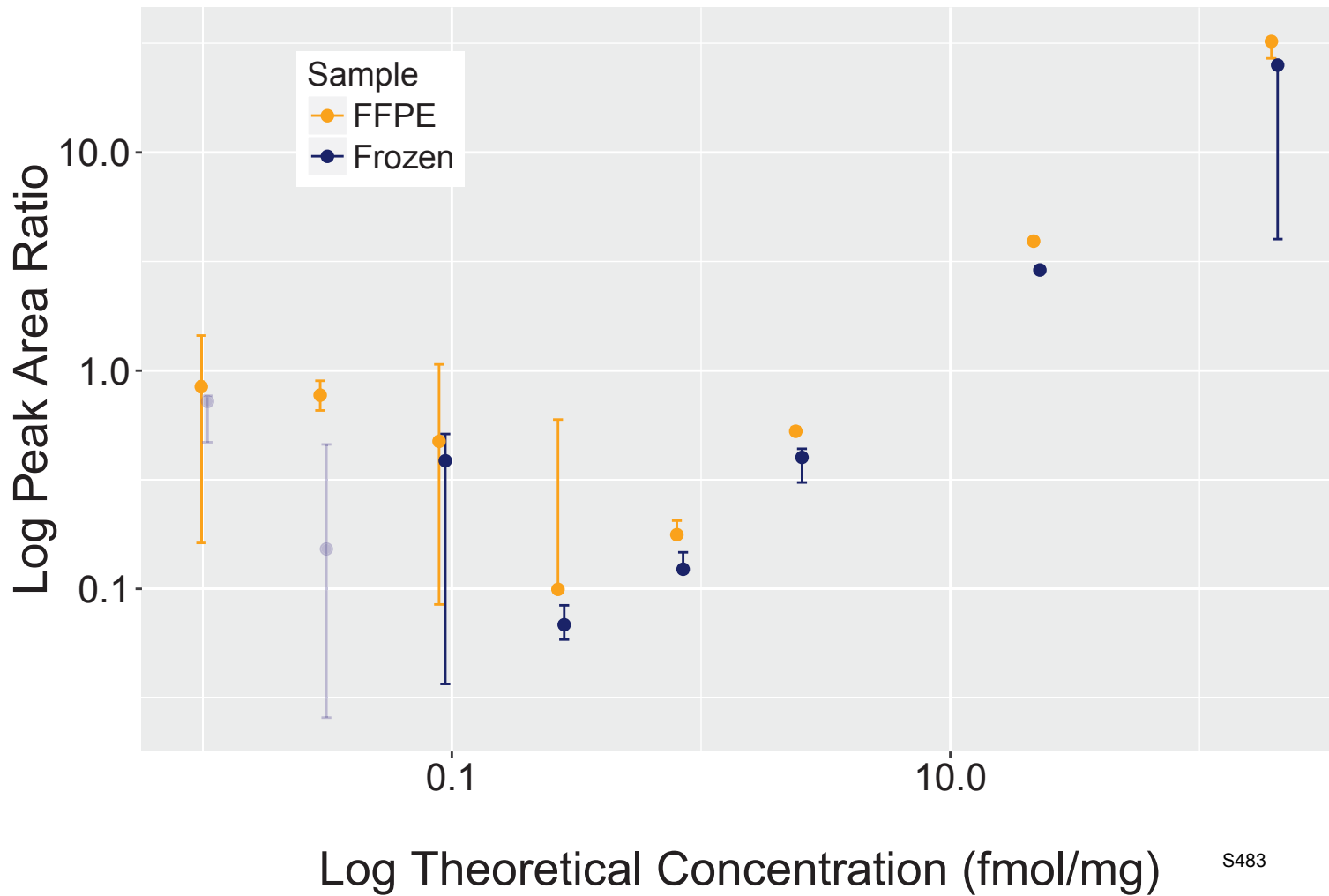
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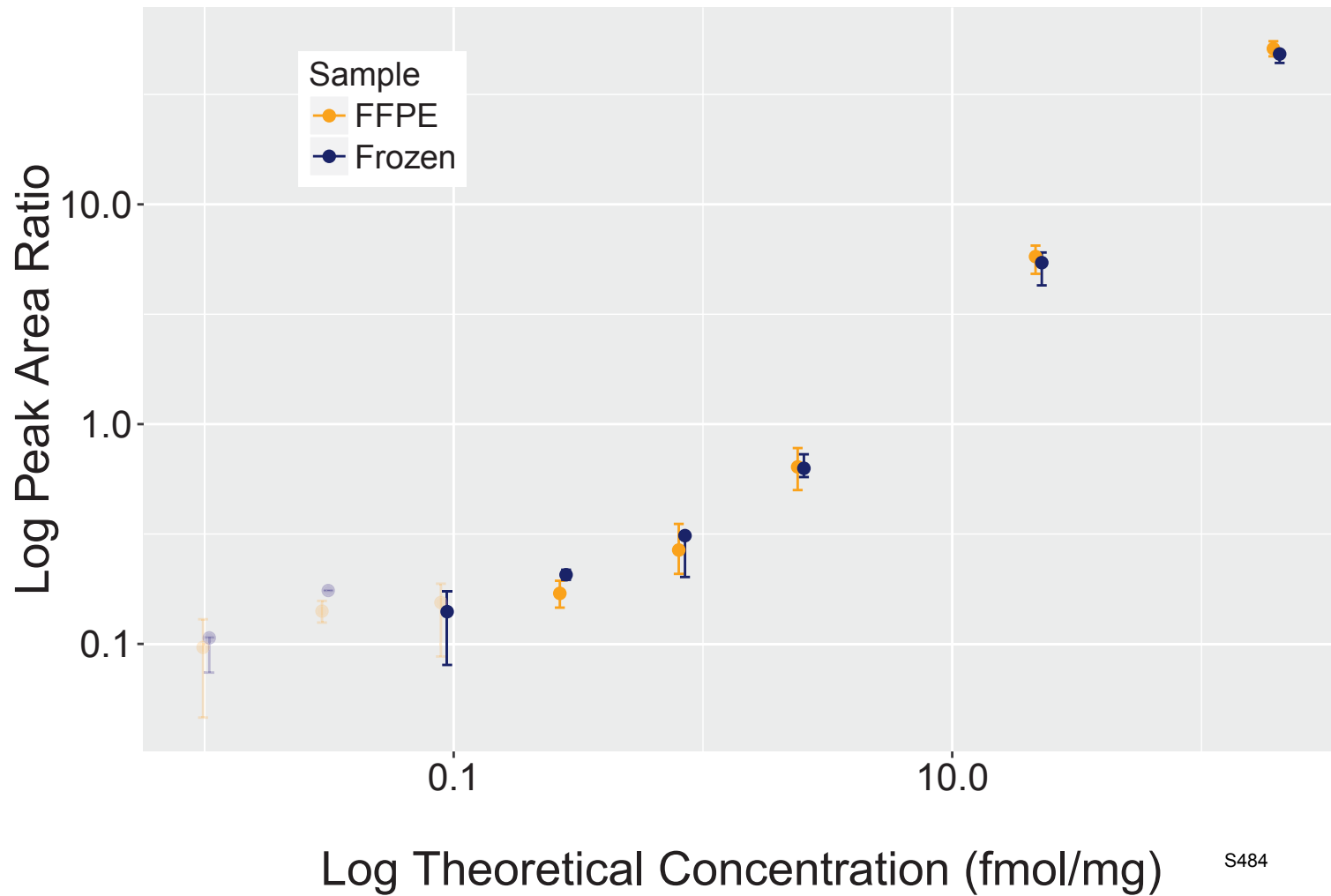
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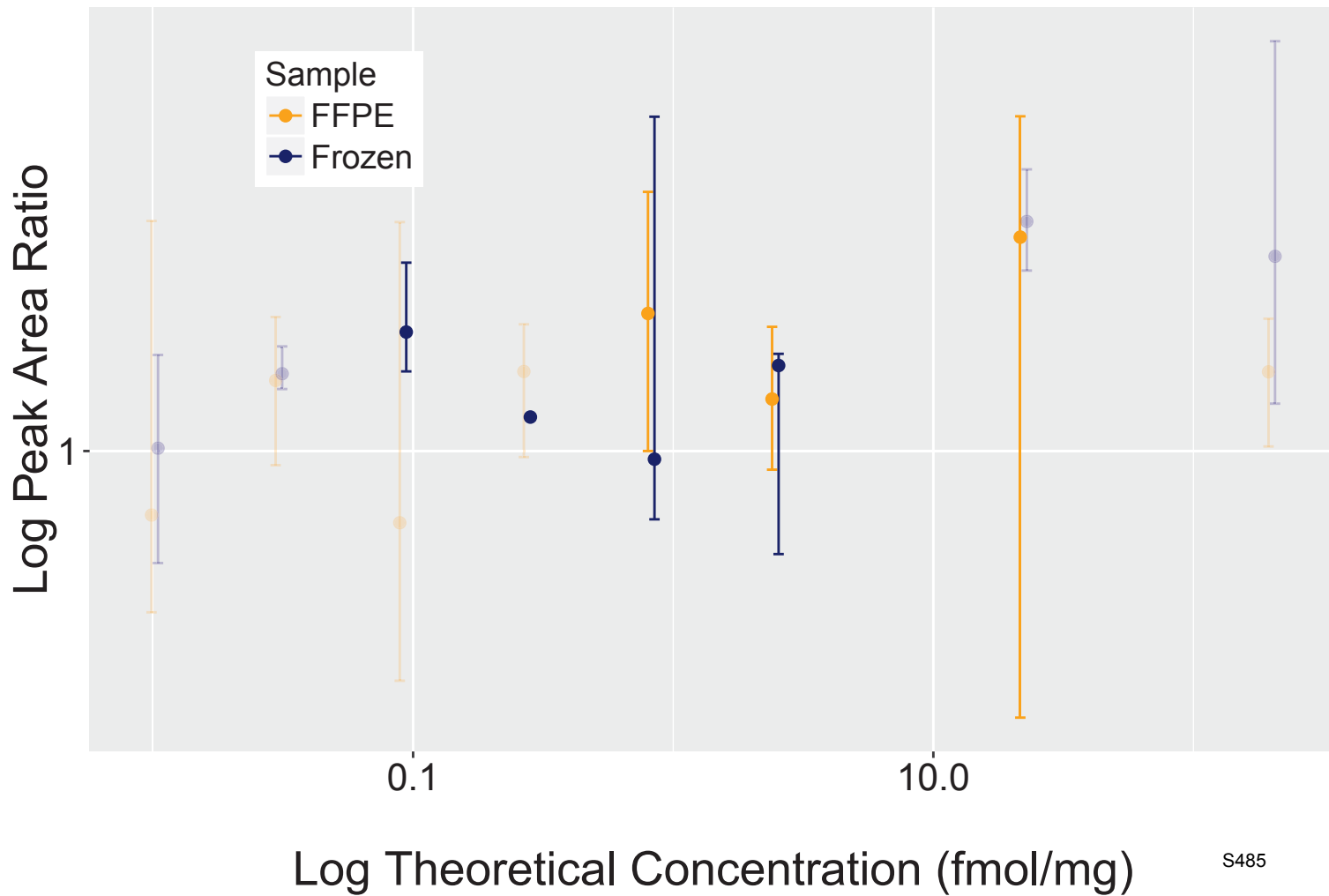
Analyte: TGM2.SEGTYC[+57]C[+57]GPVPVR



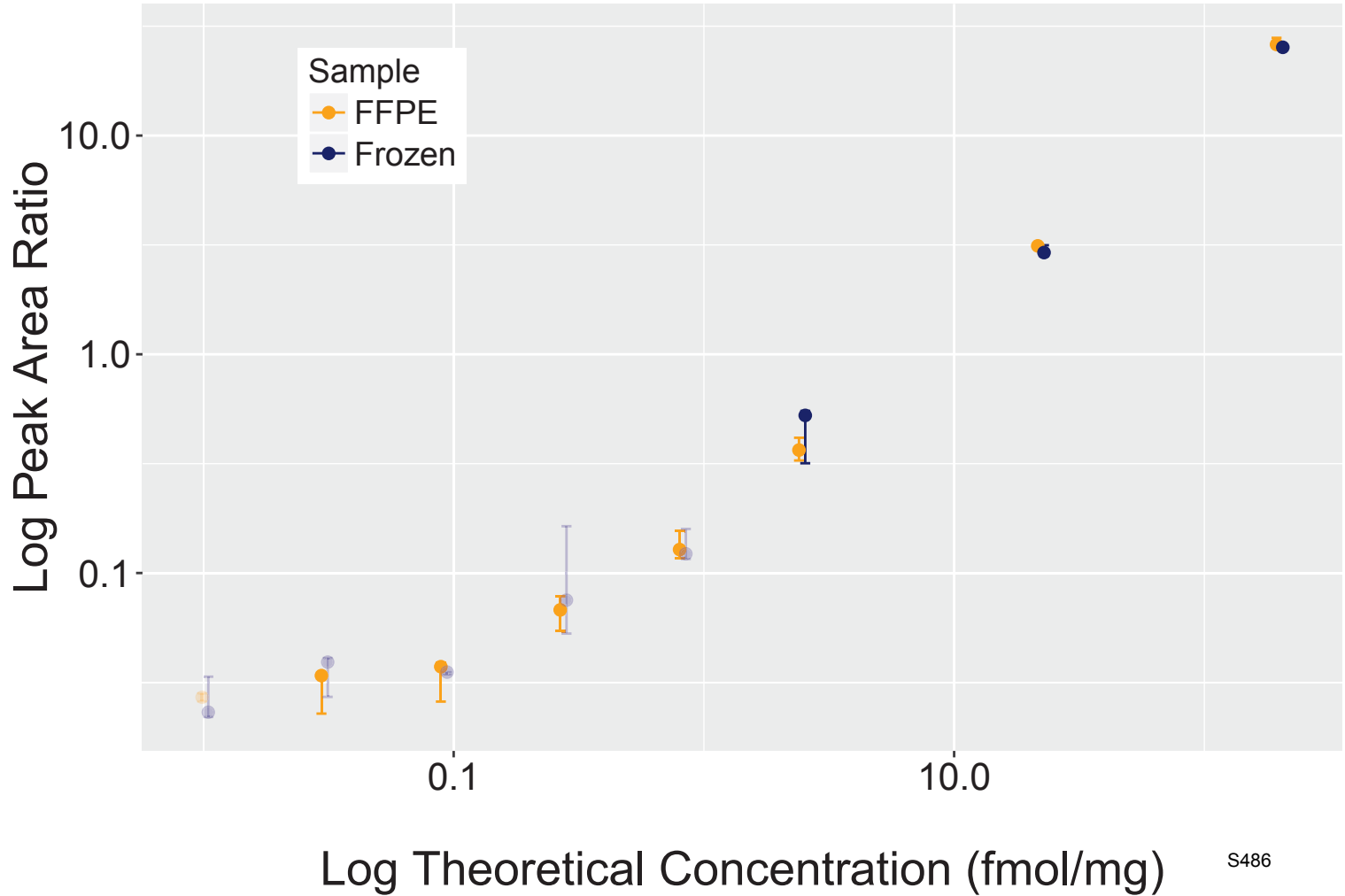
Analyte: DBN1.SESEVEEAAIIAQRPDNP



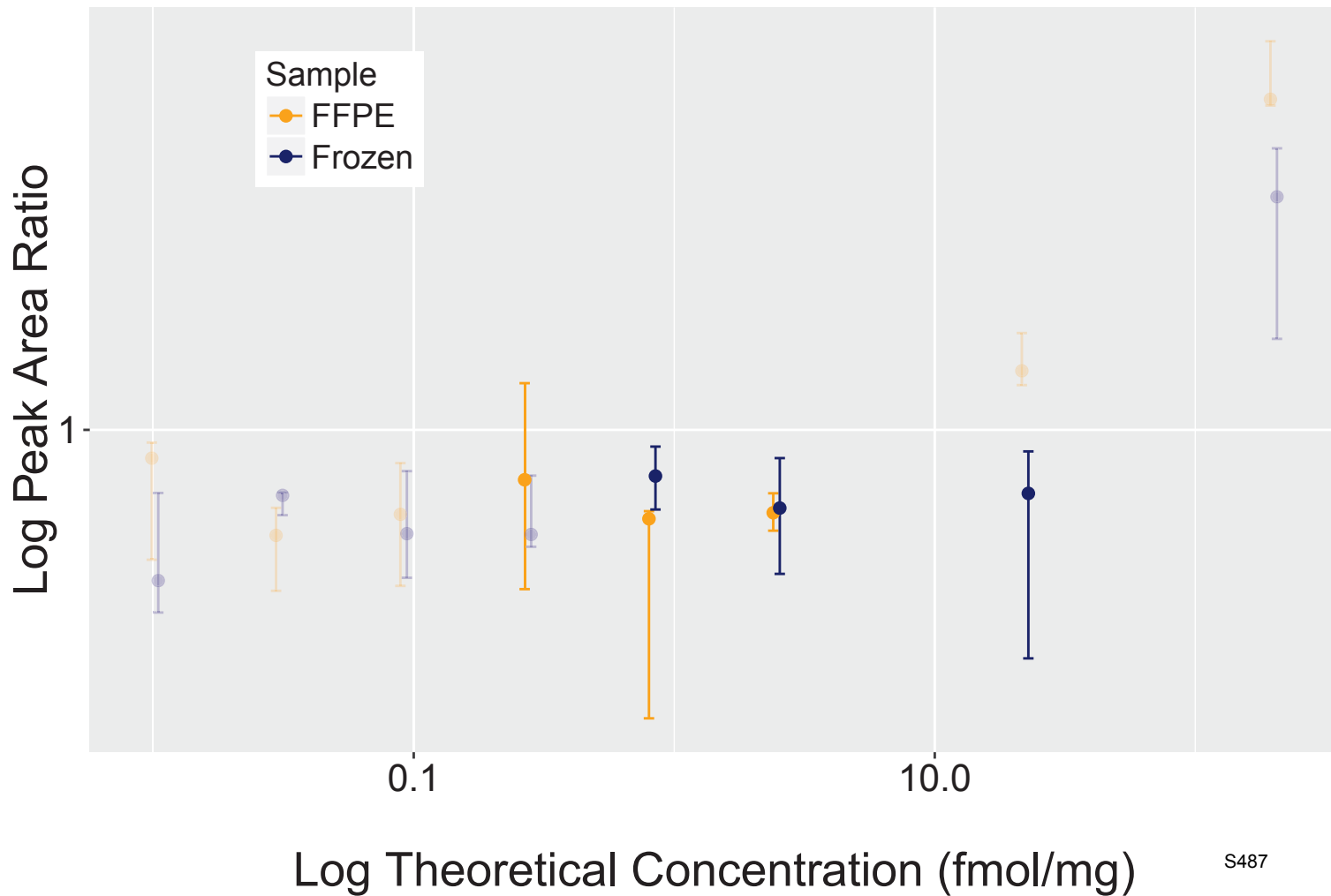
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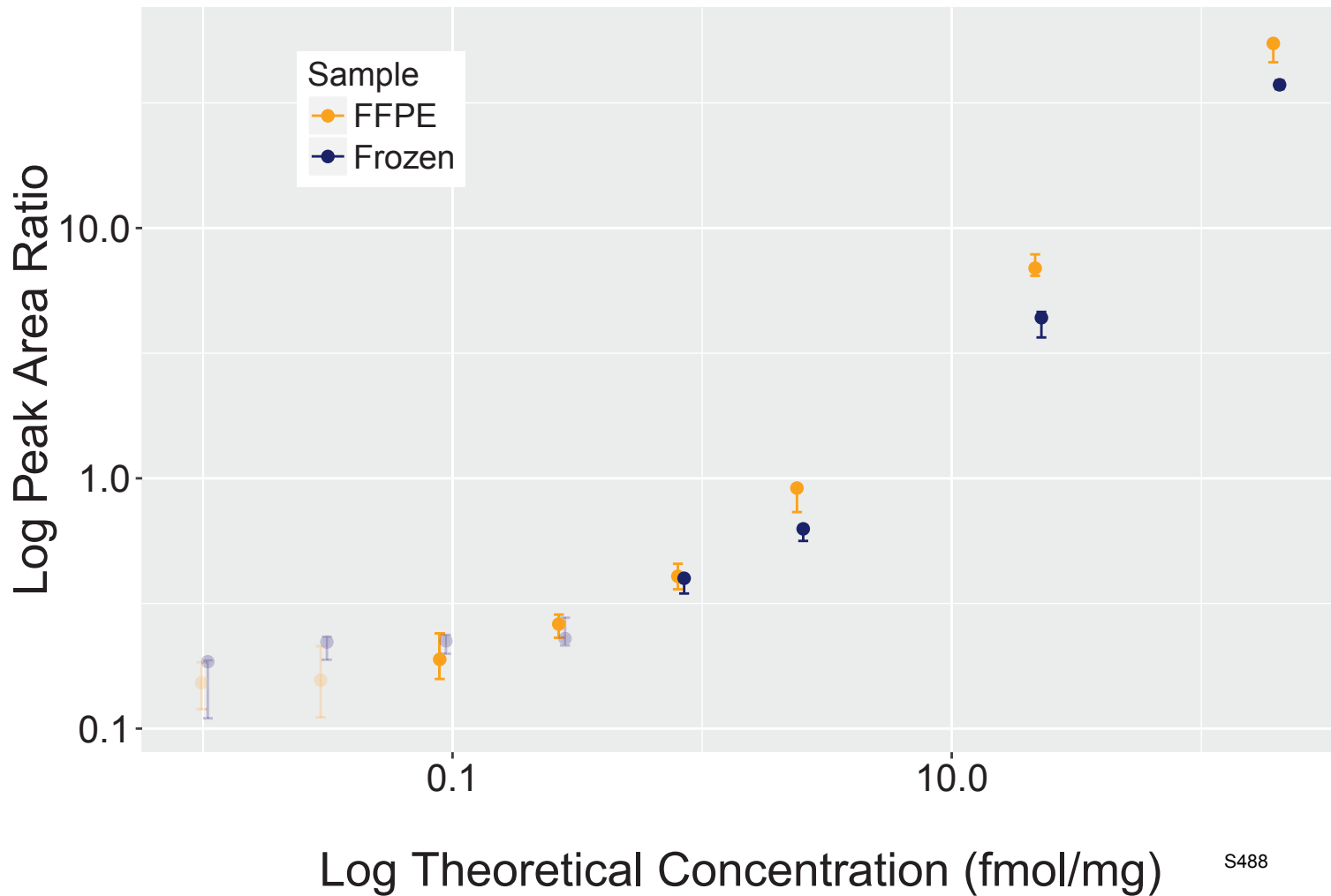
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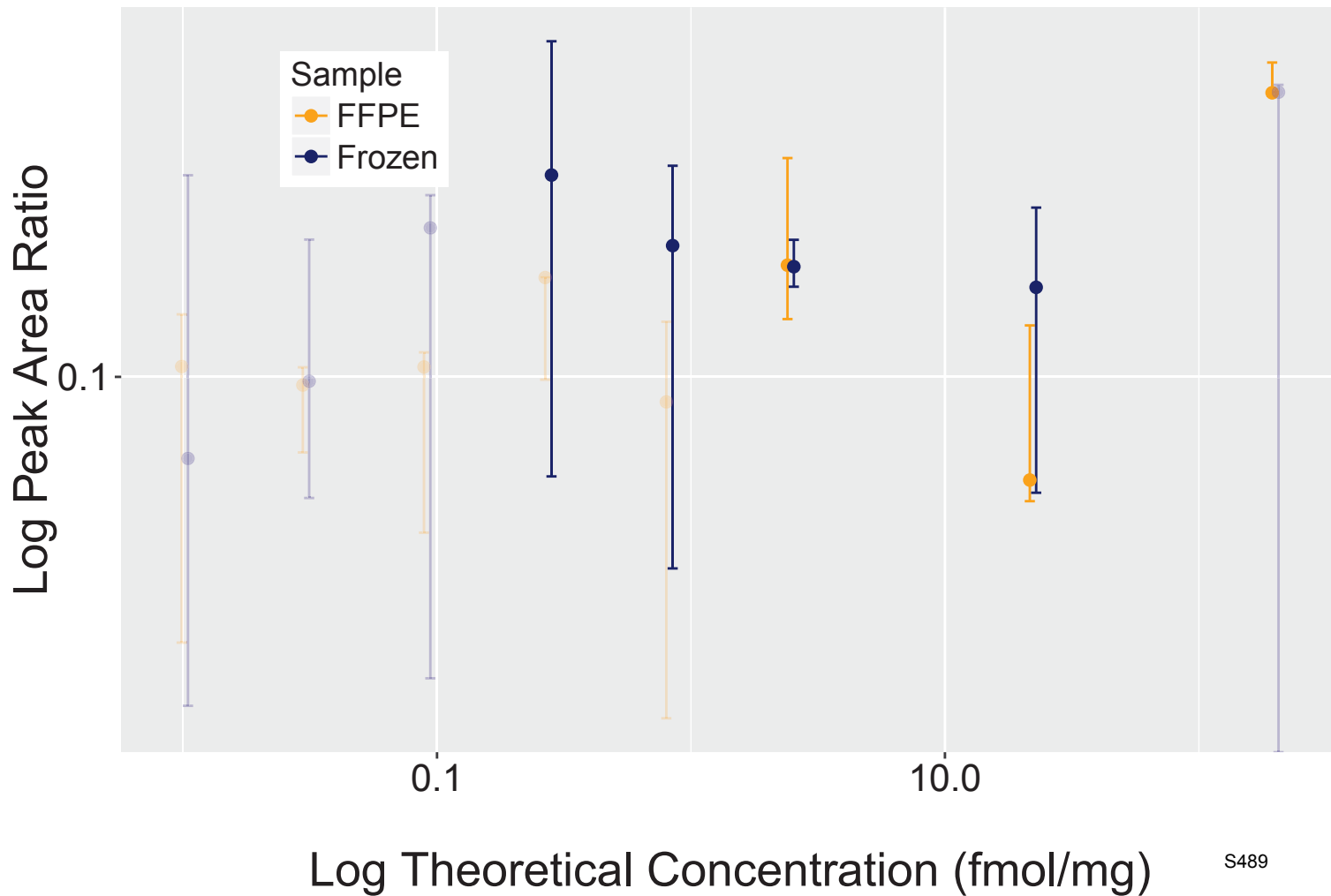
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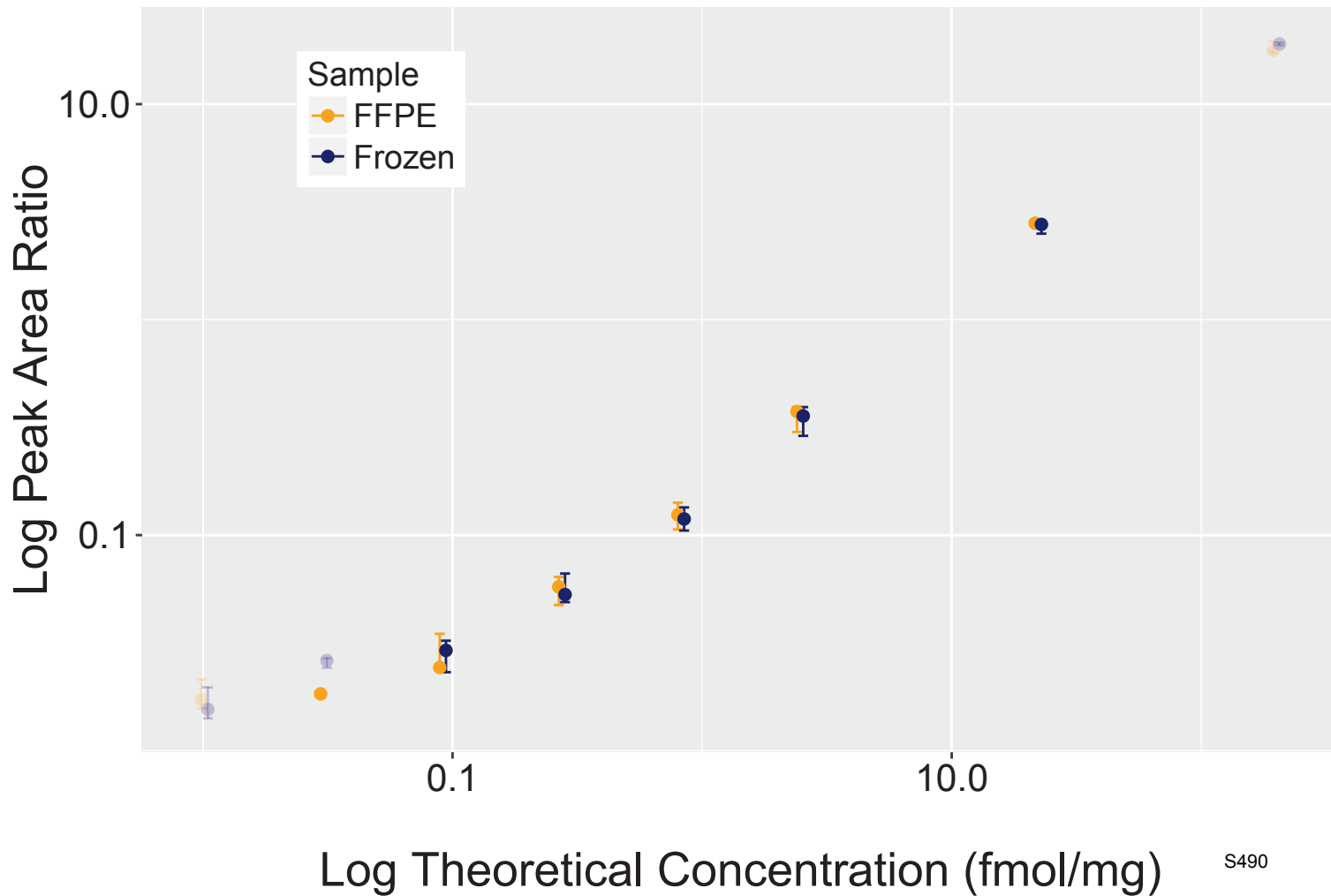
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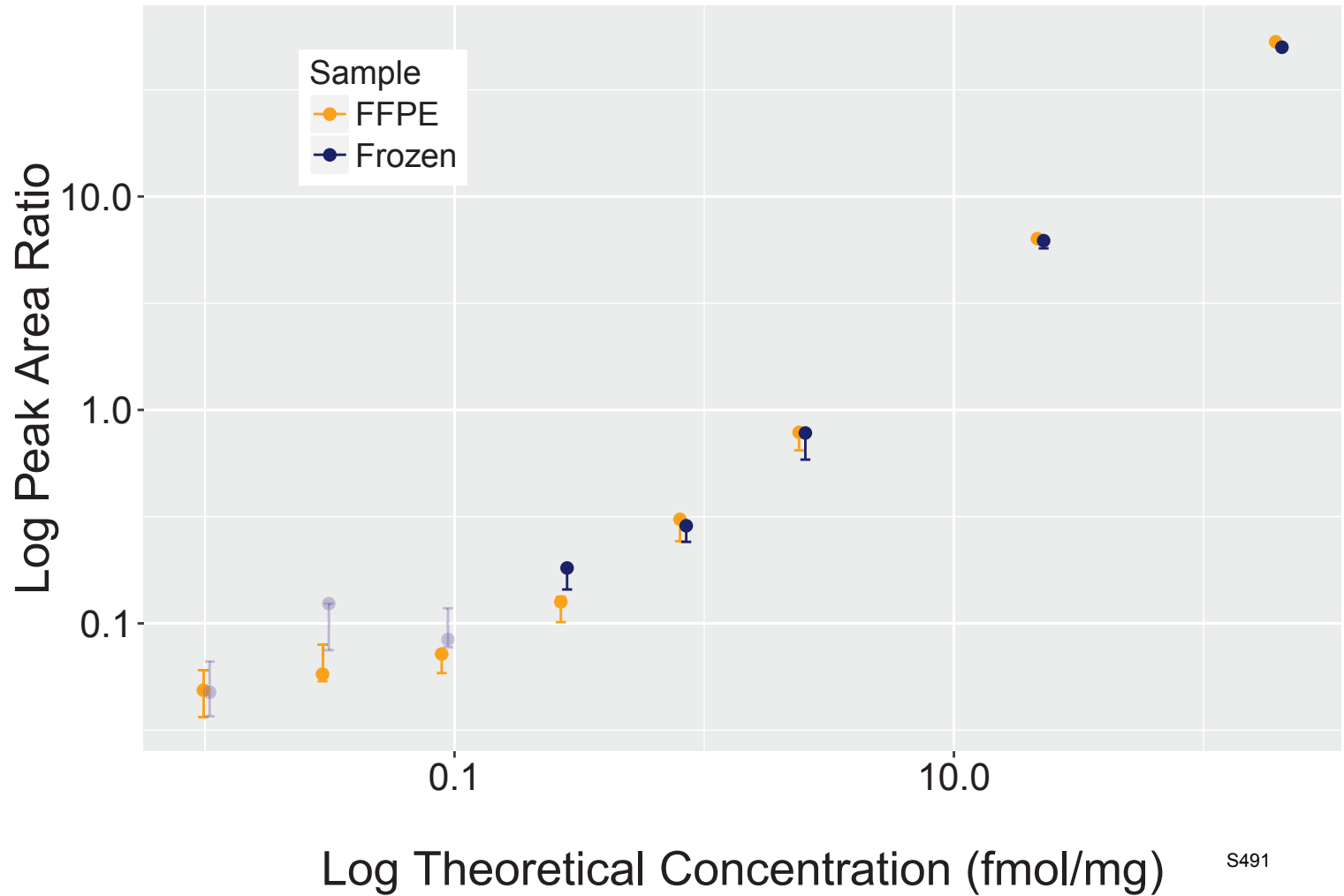
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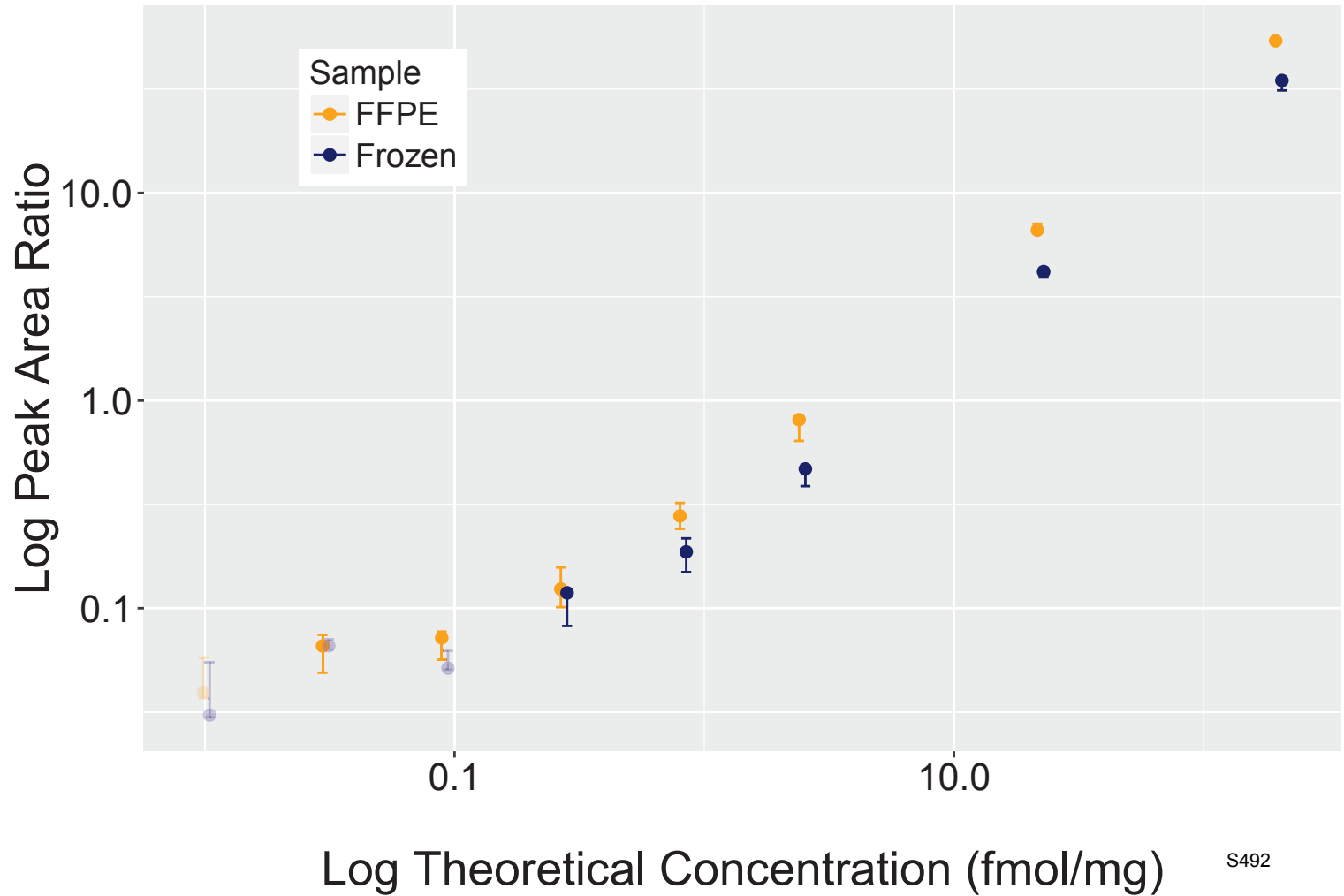
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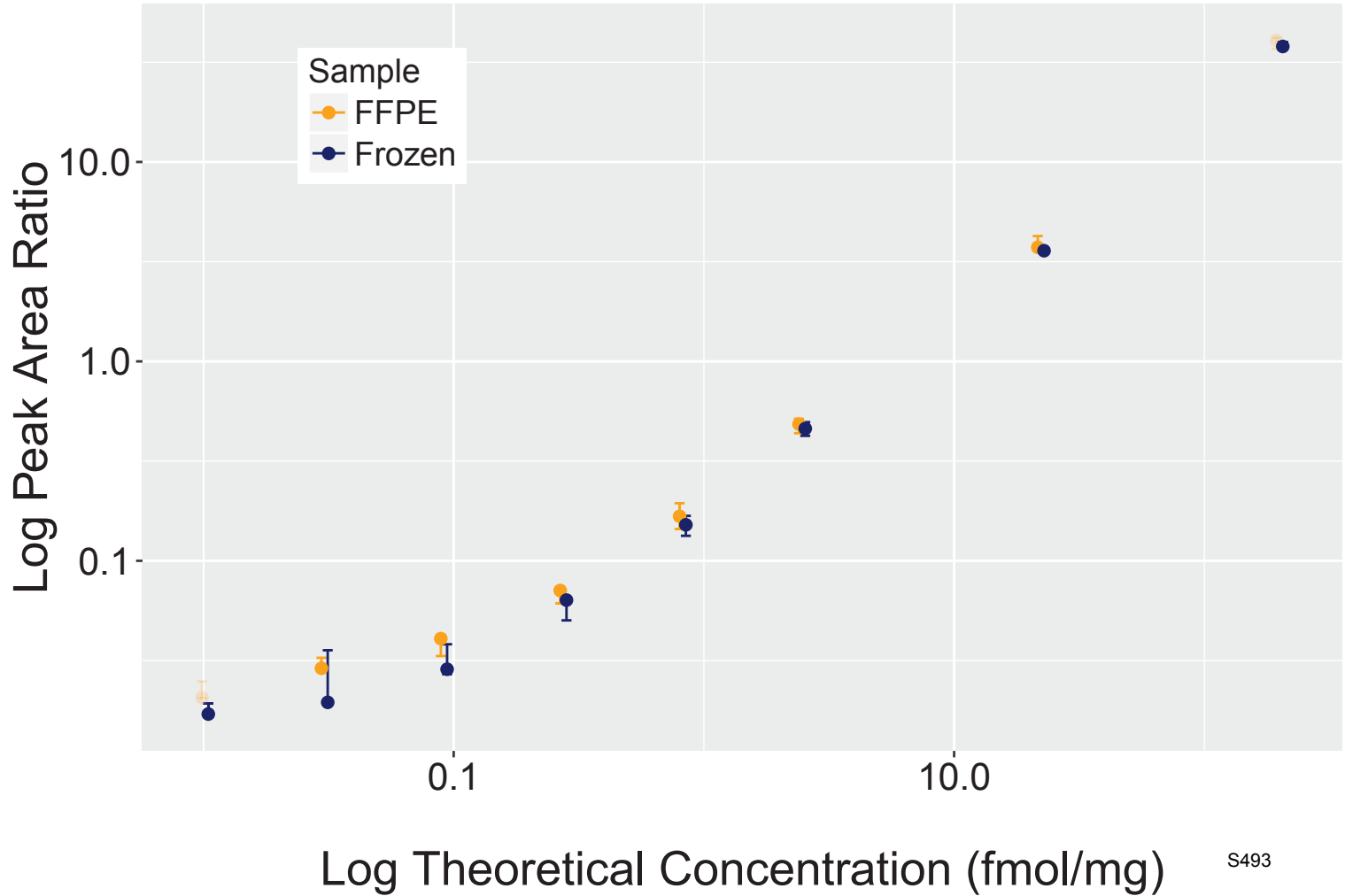
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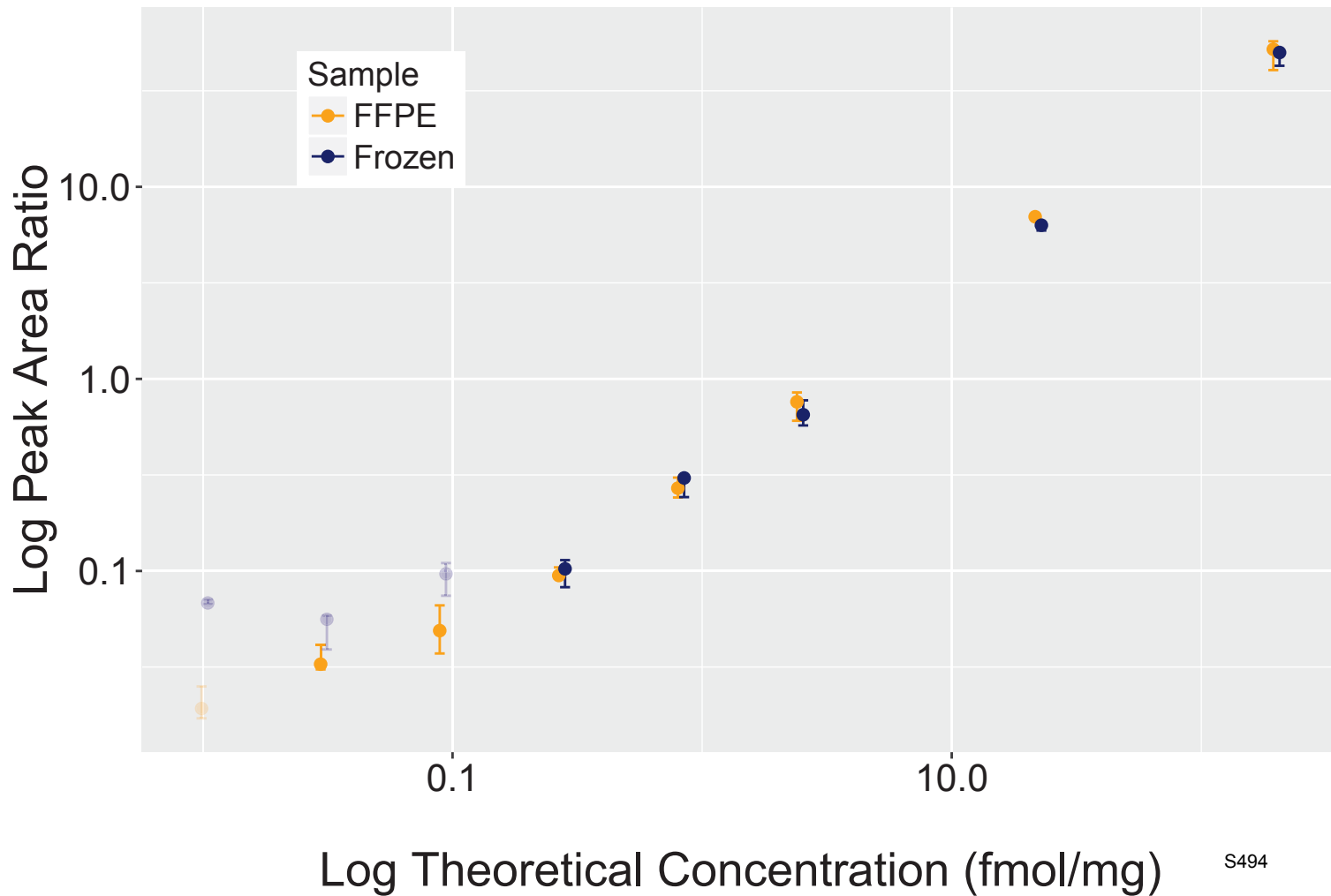
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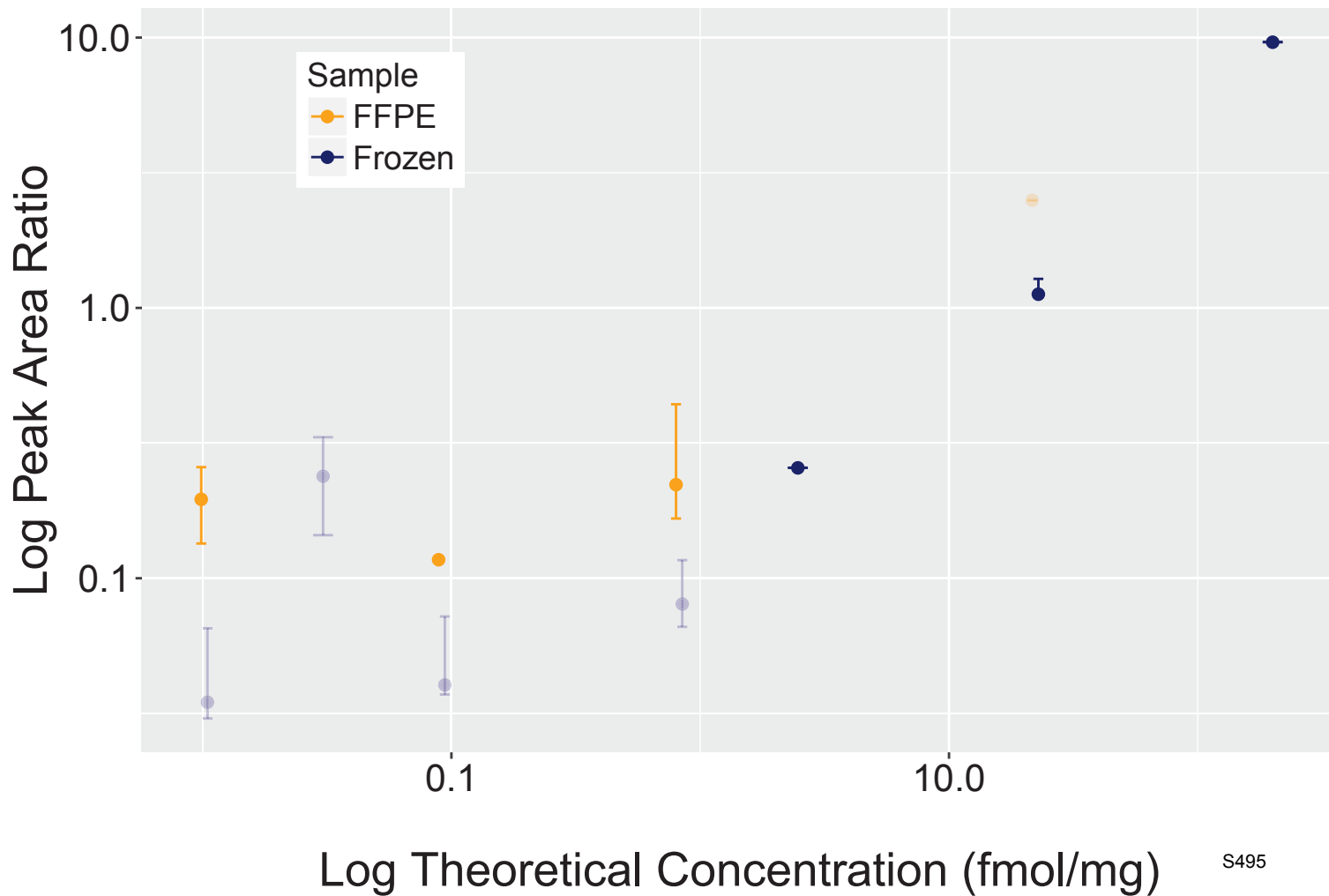
Analyte: LANCL2.SGNYPSSLNETDR



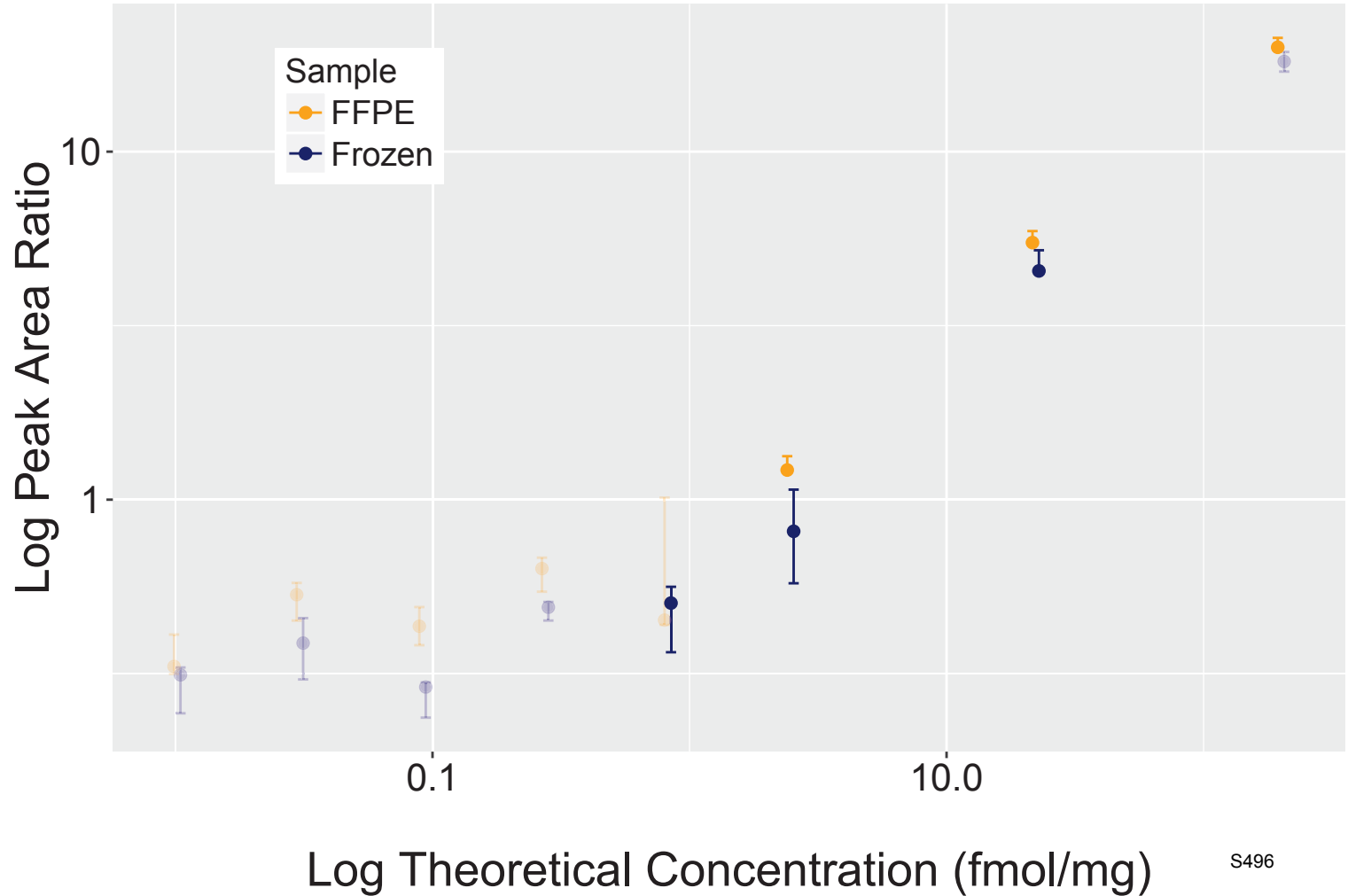
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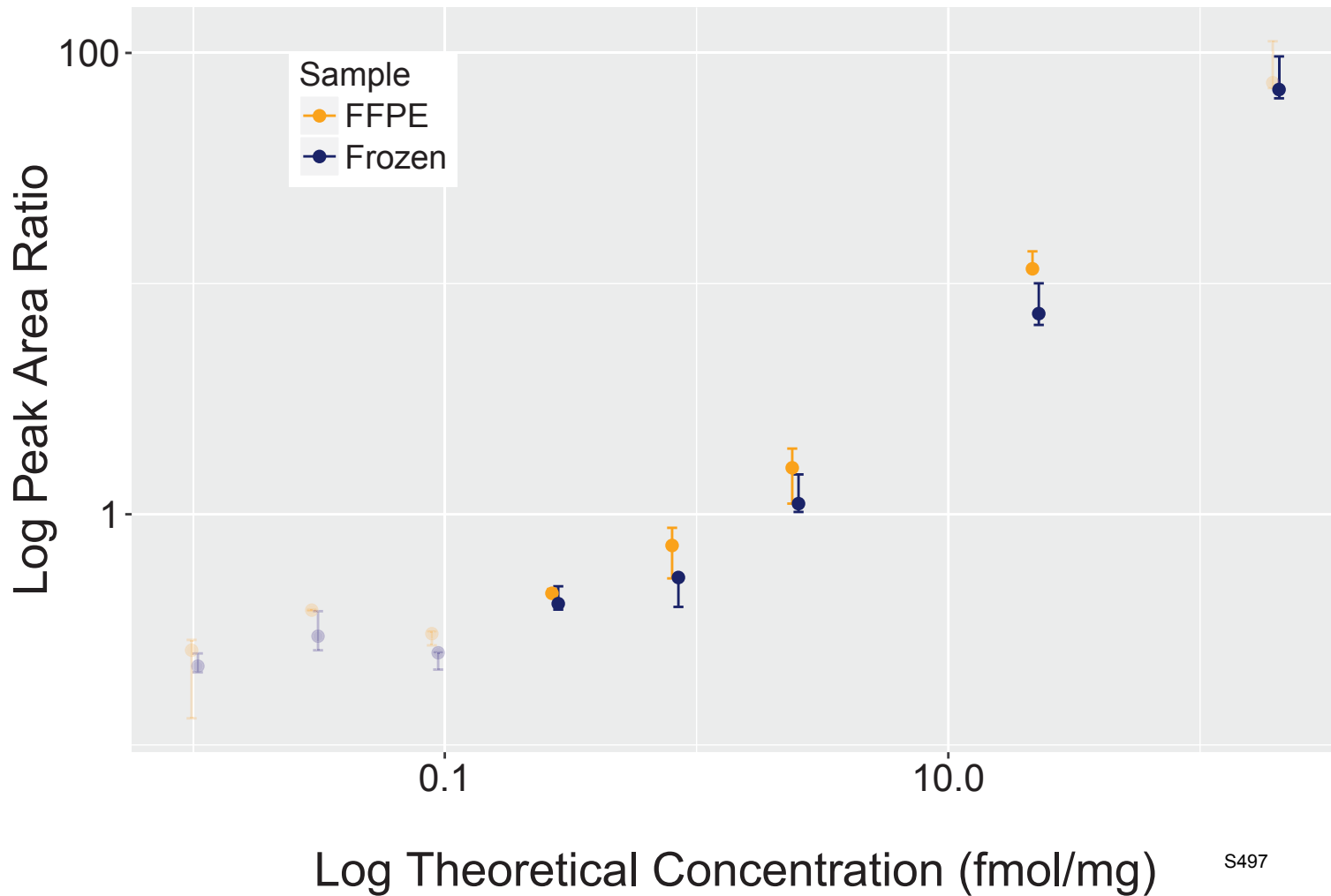
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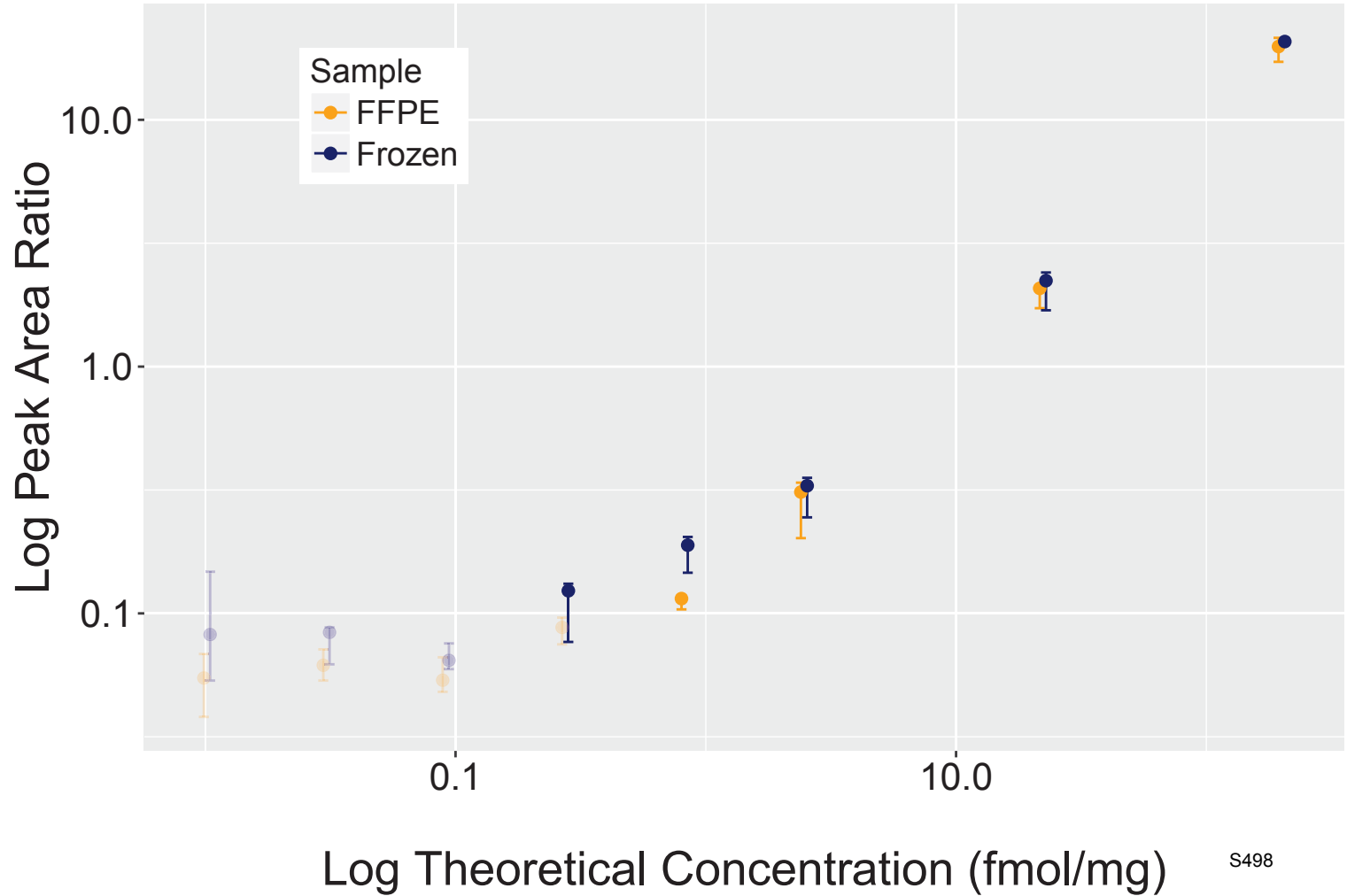
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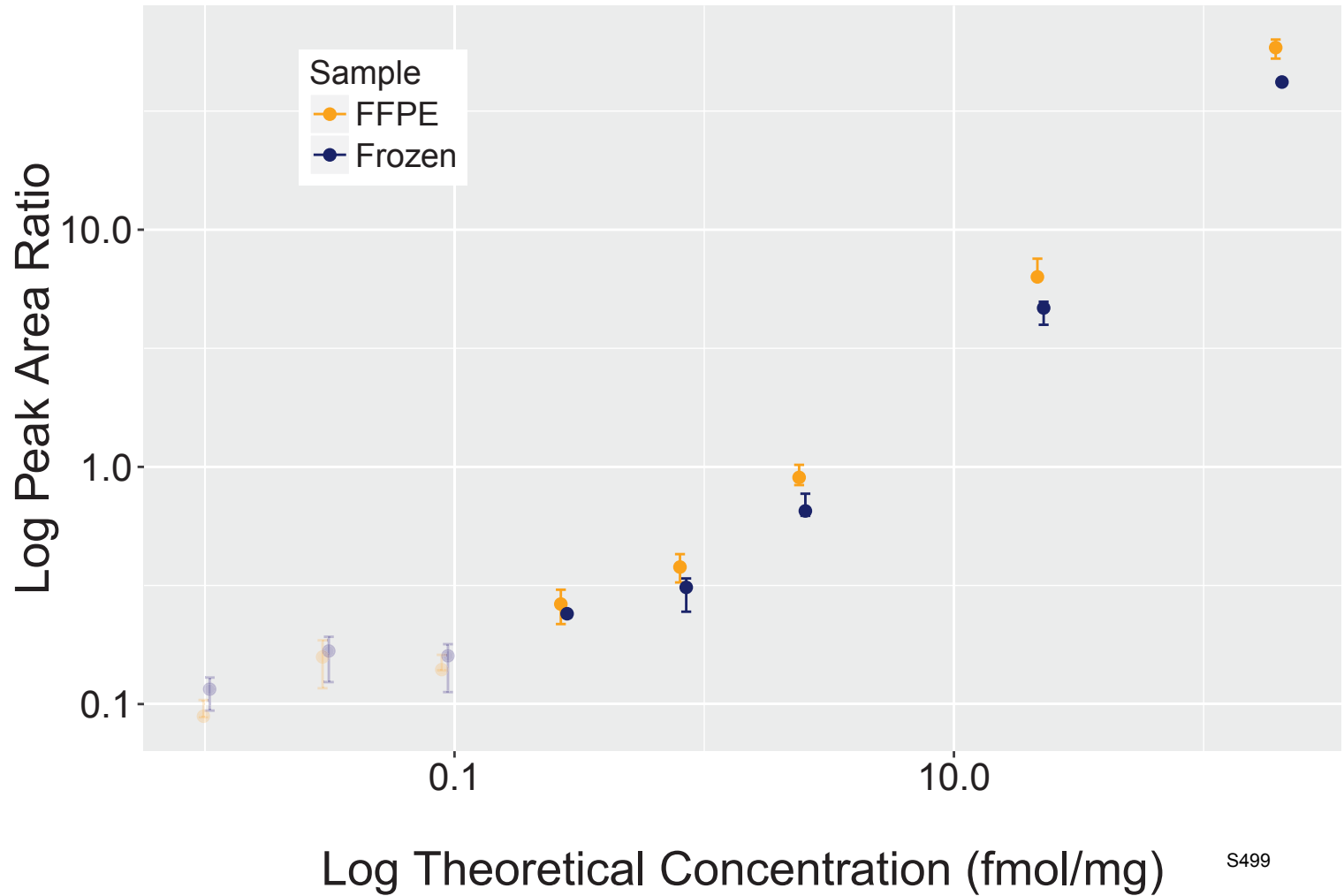
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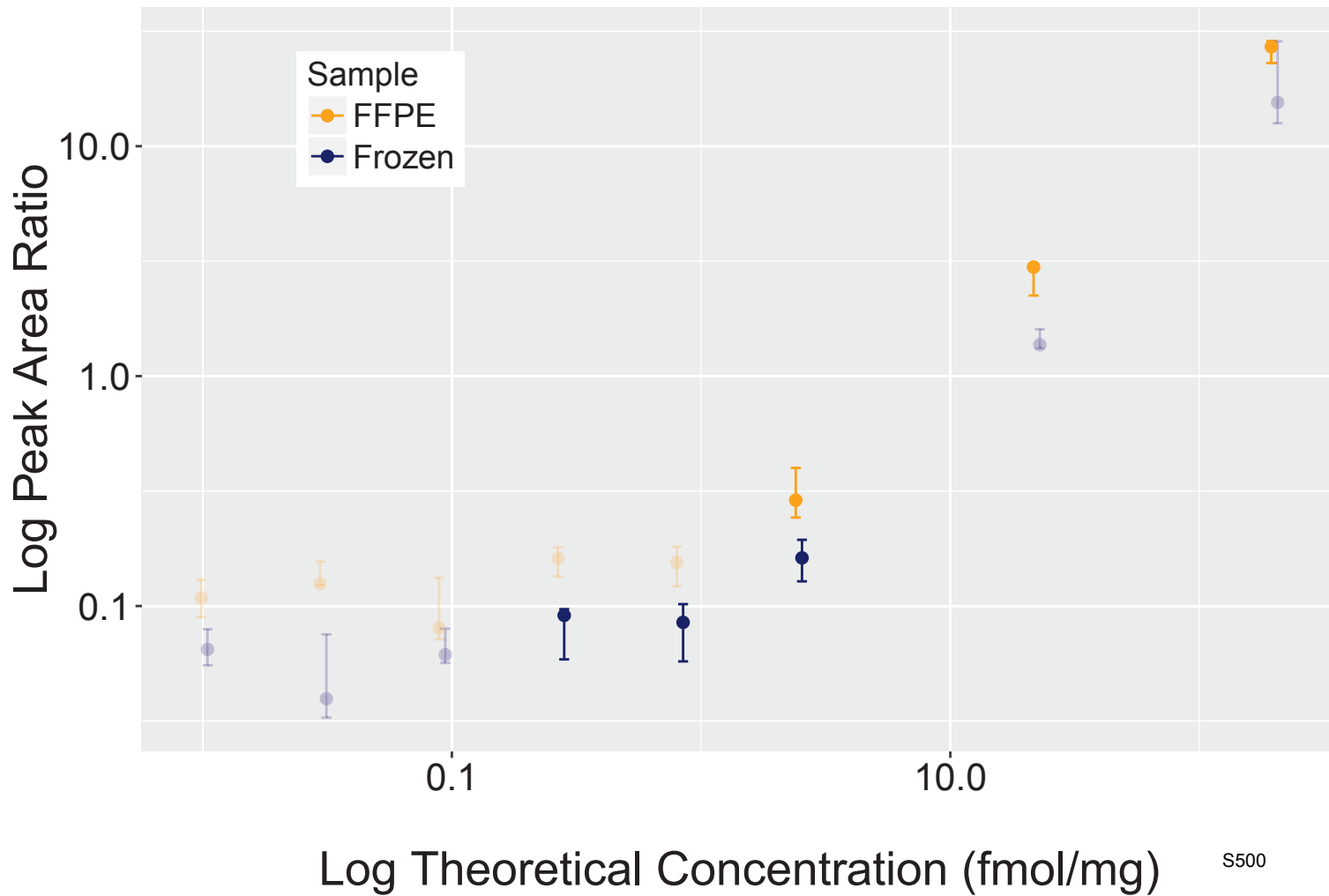
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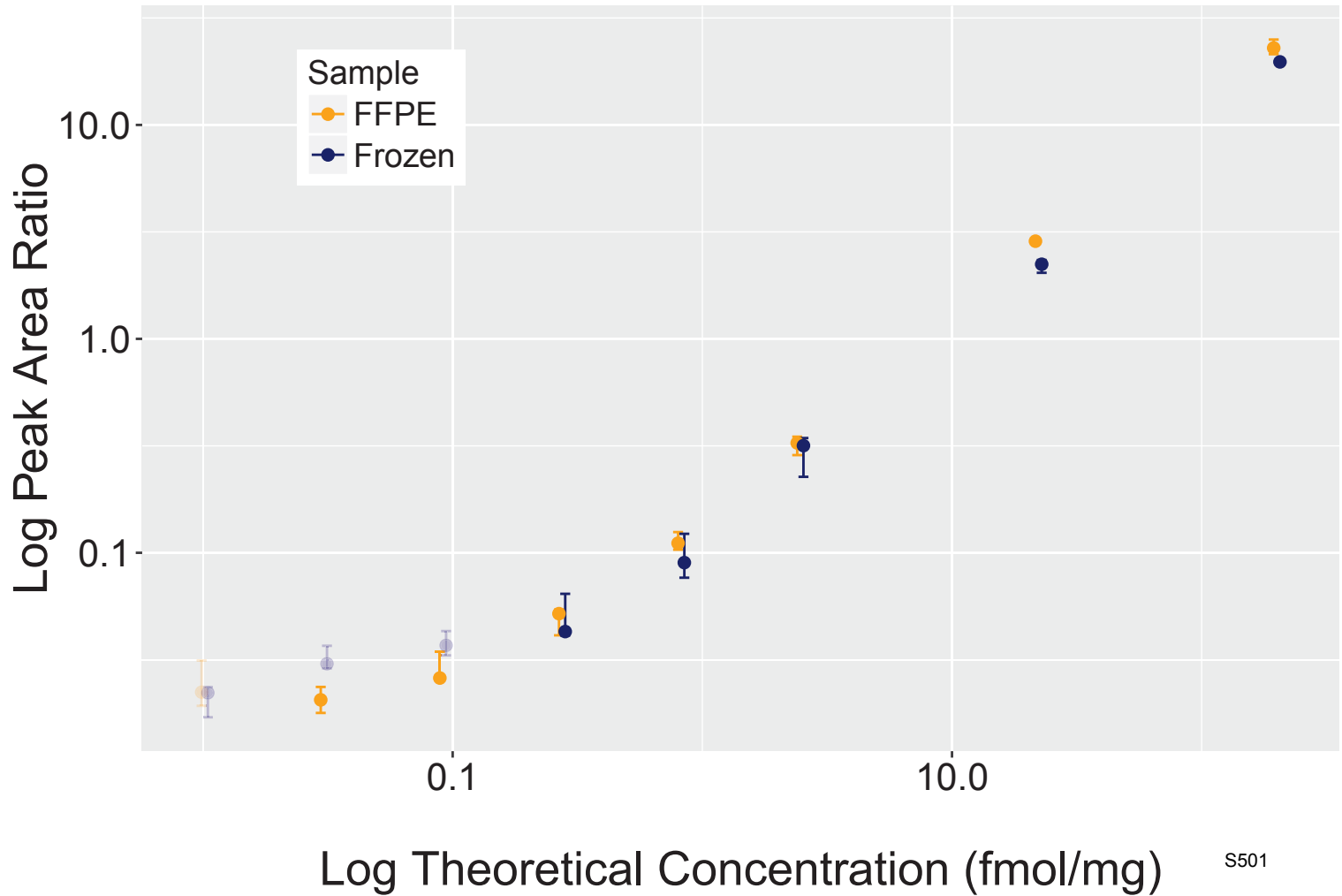
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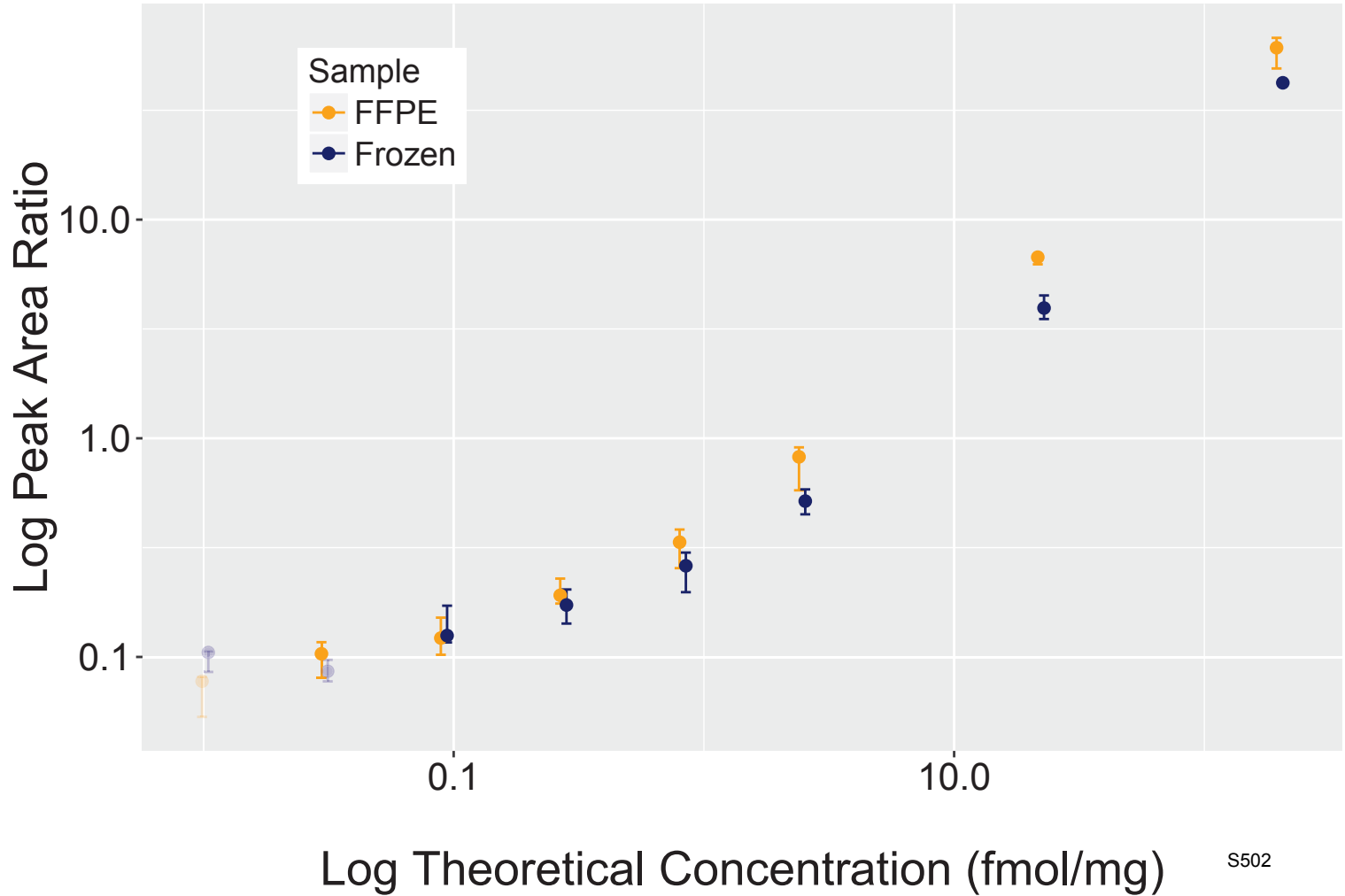
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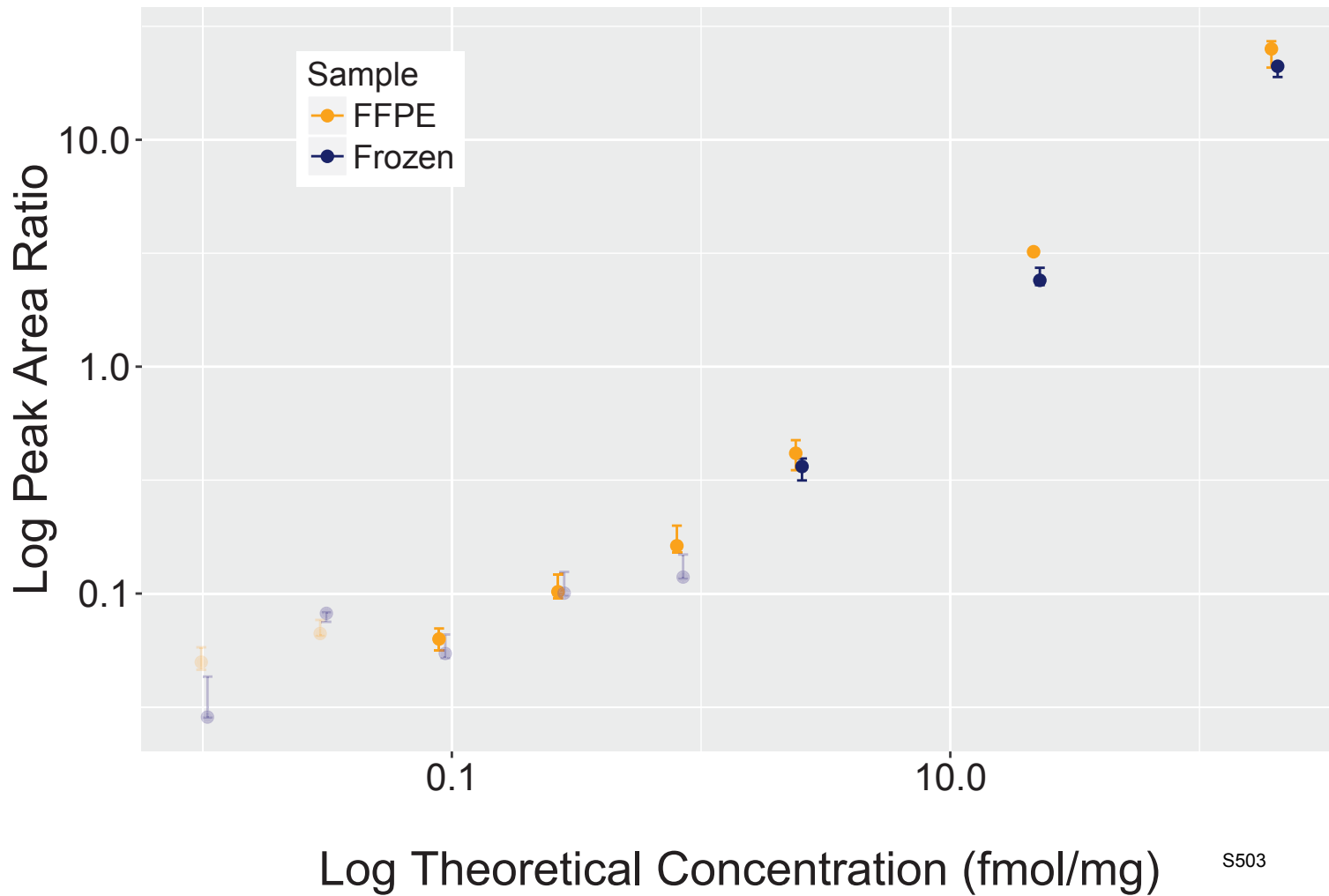
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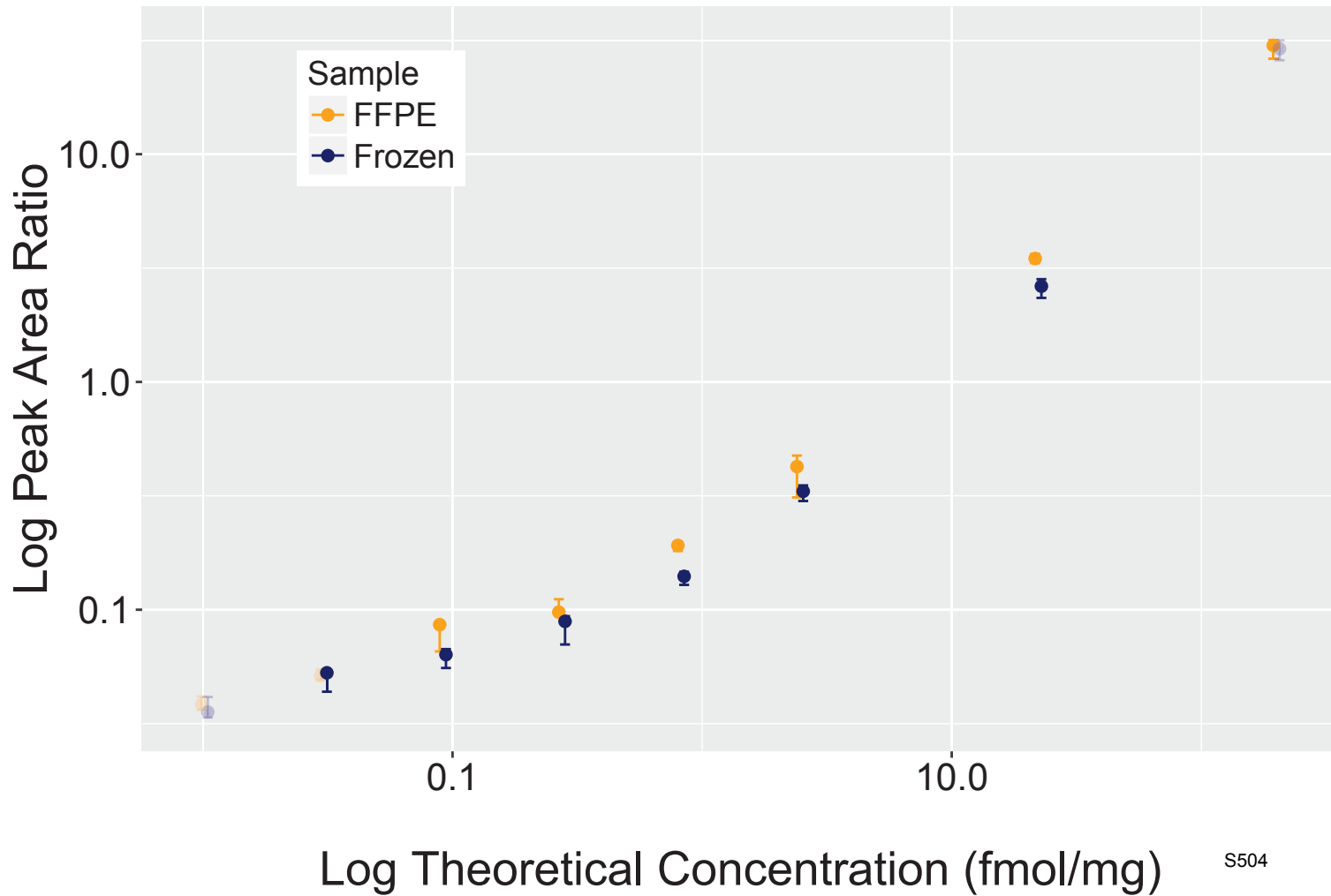
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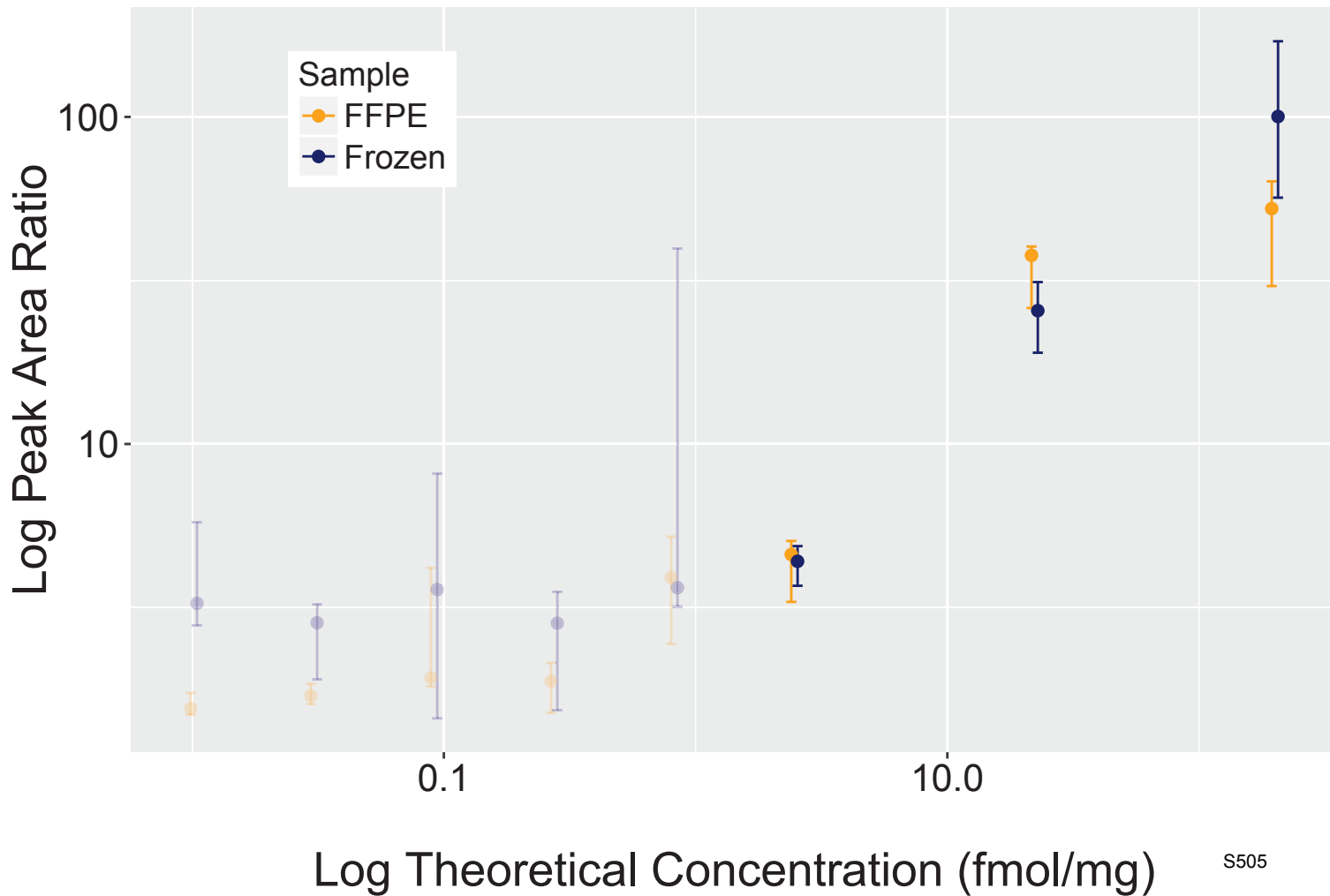
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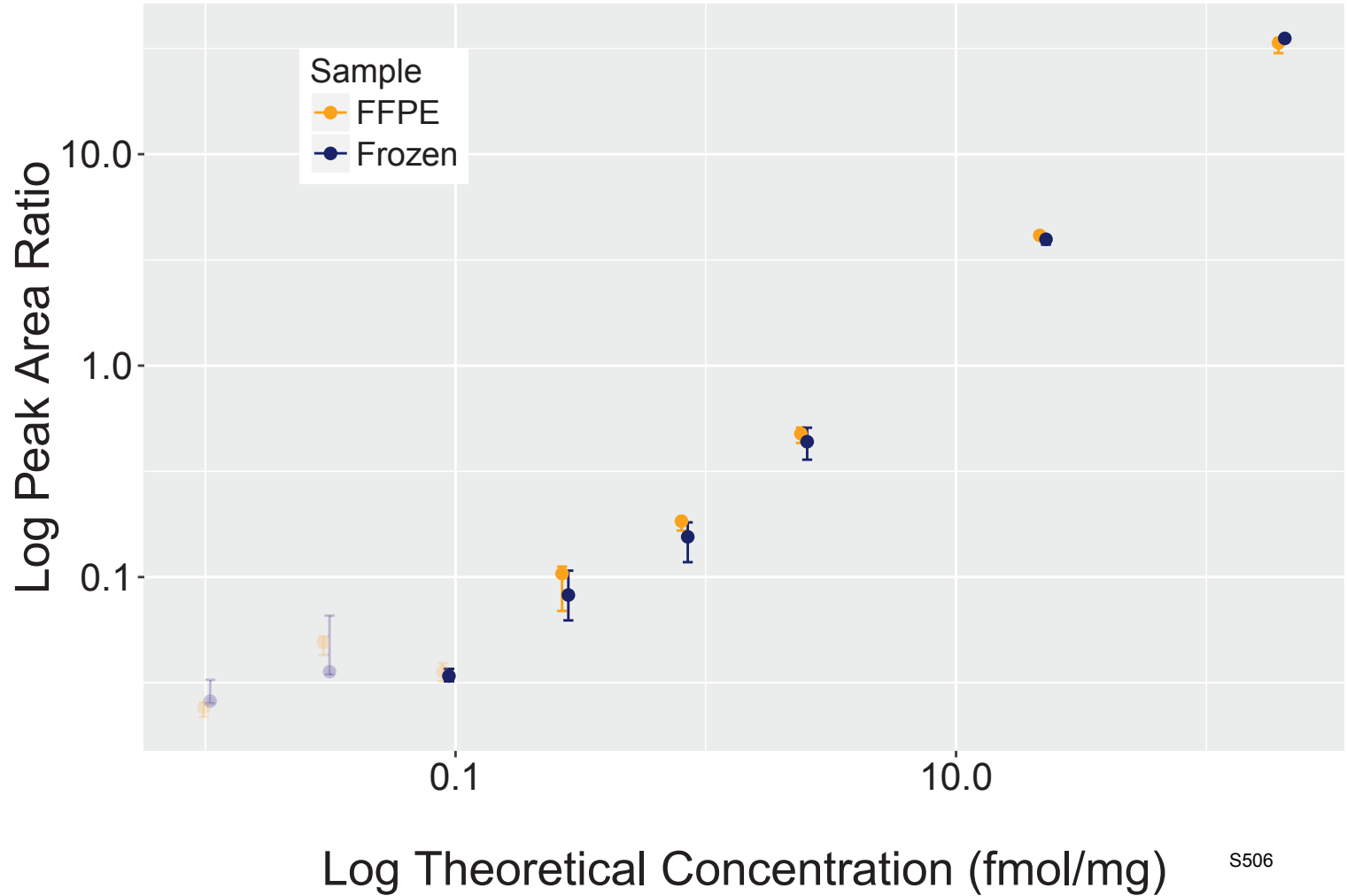
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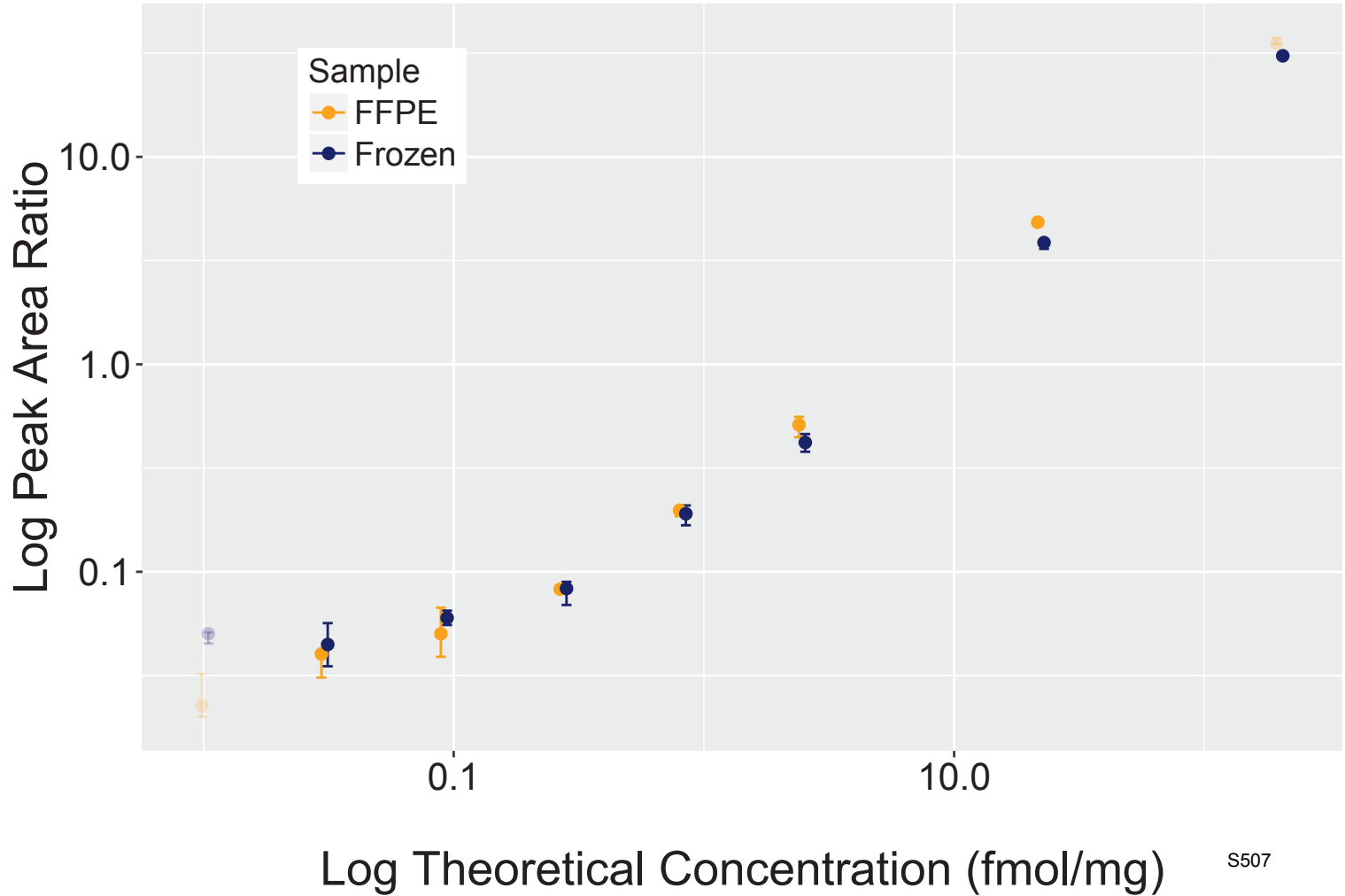
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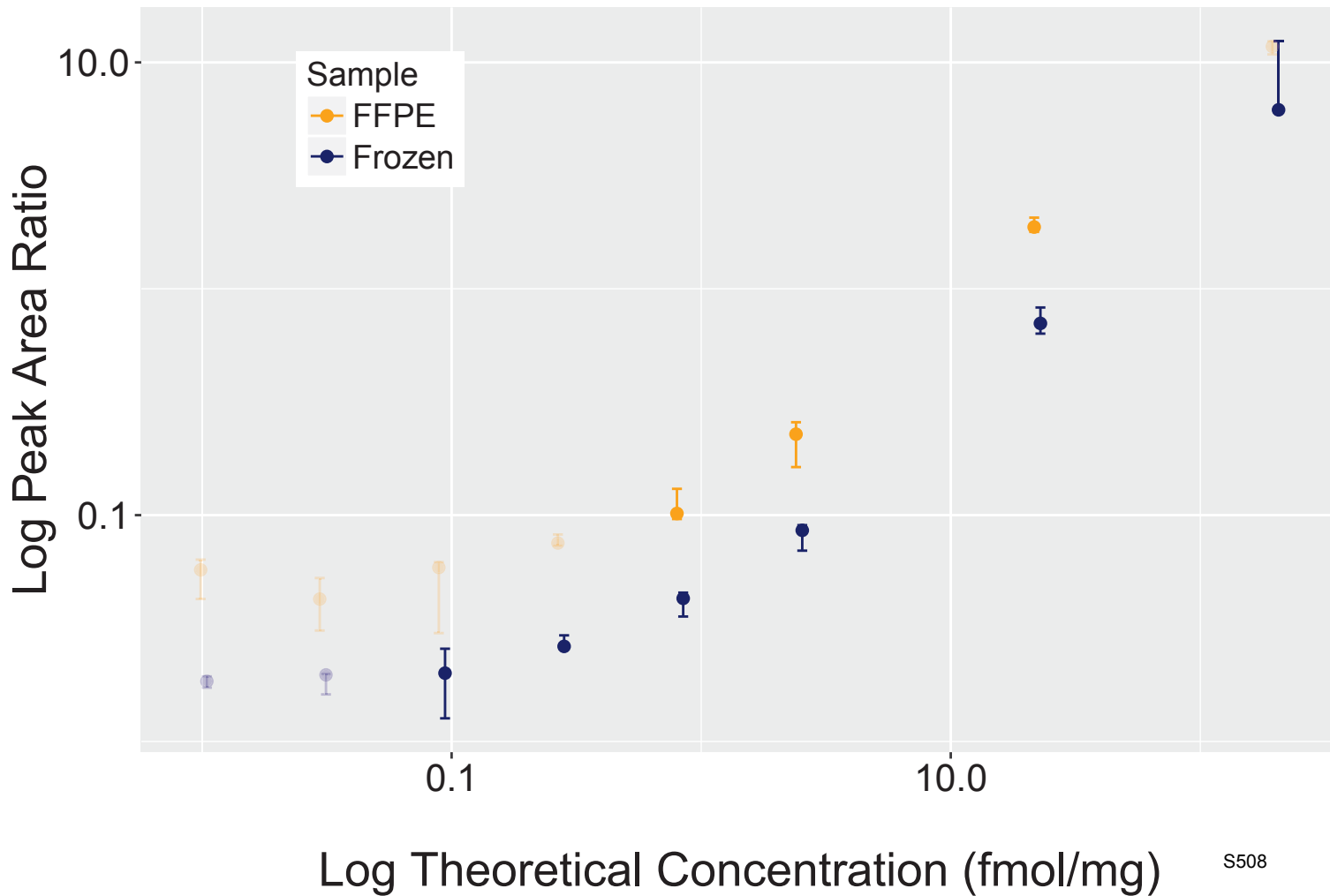
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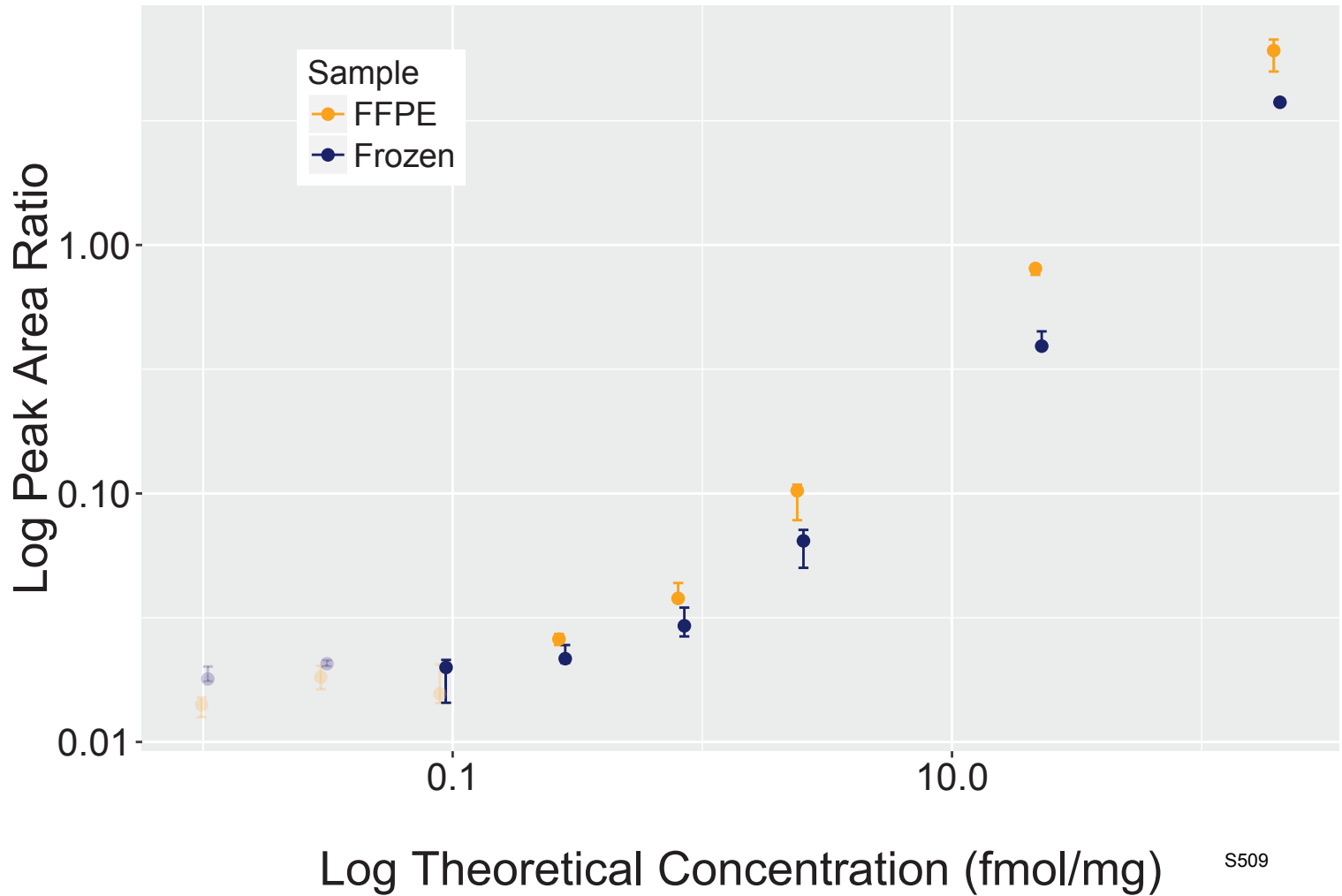
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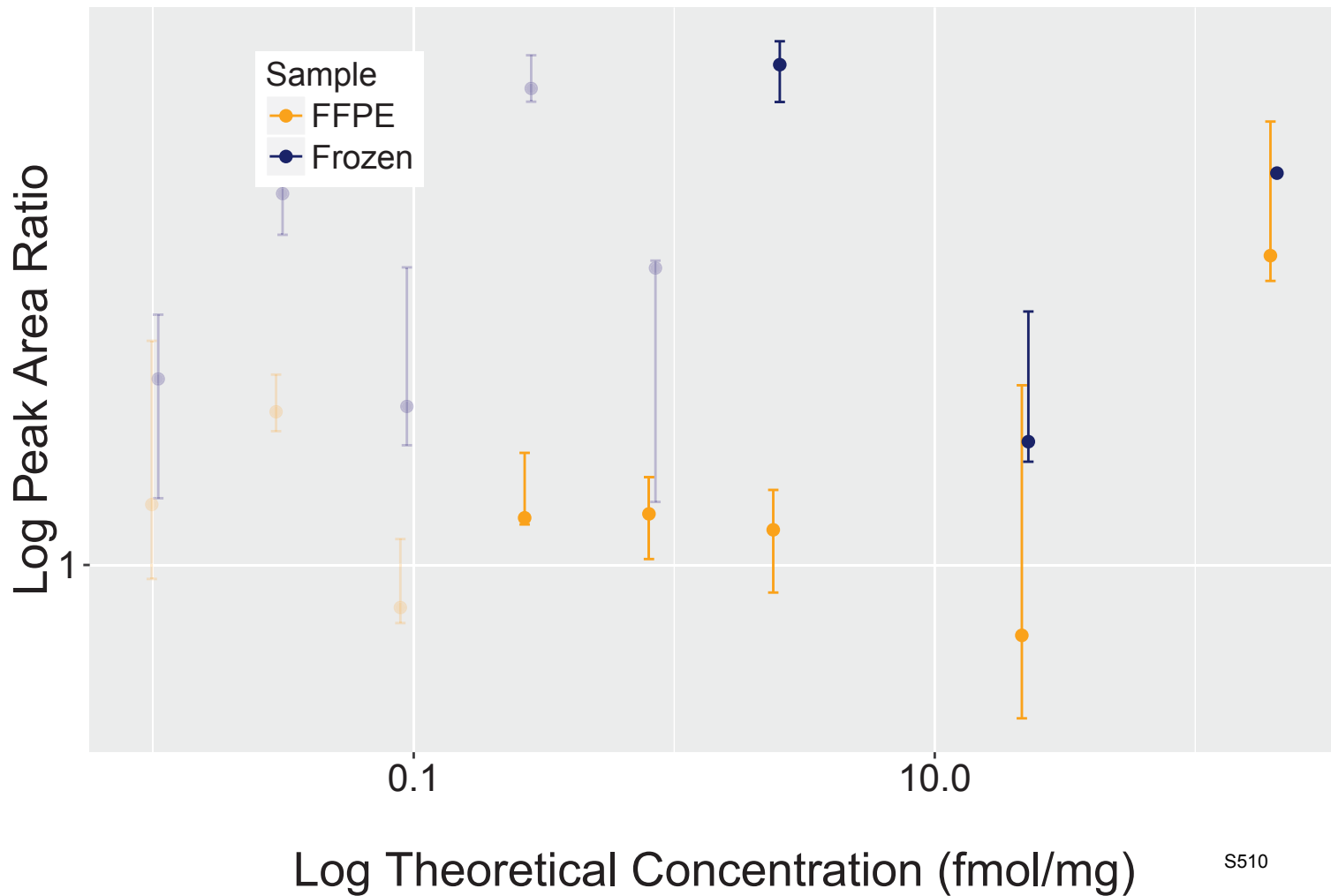
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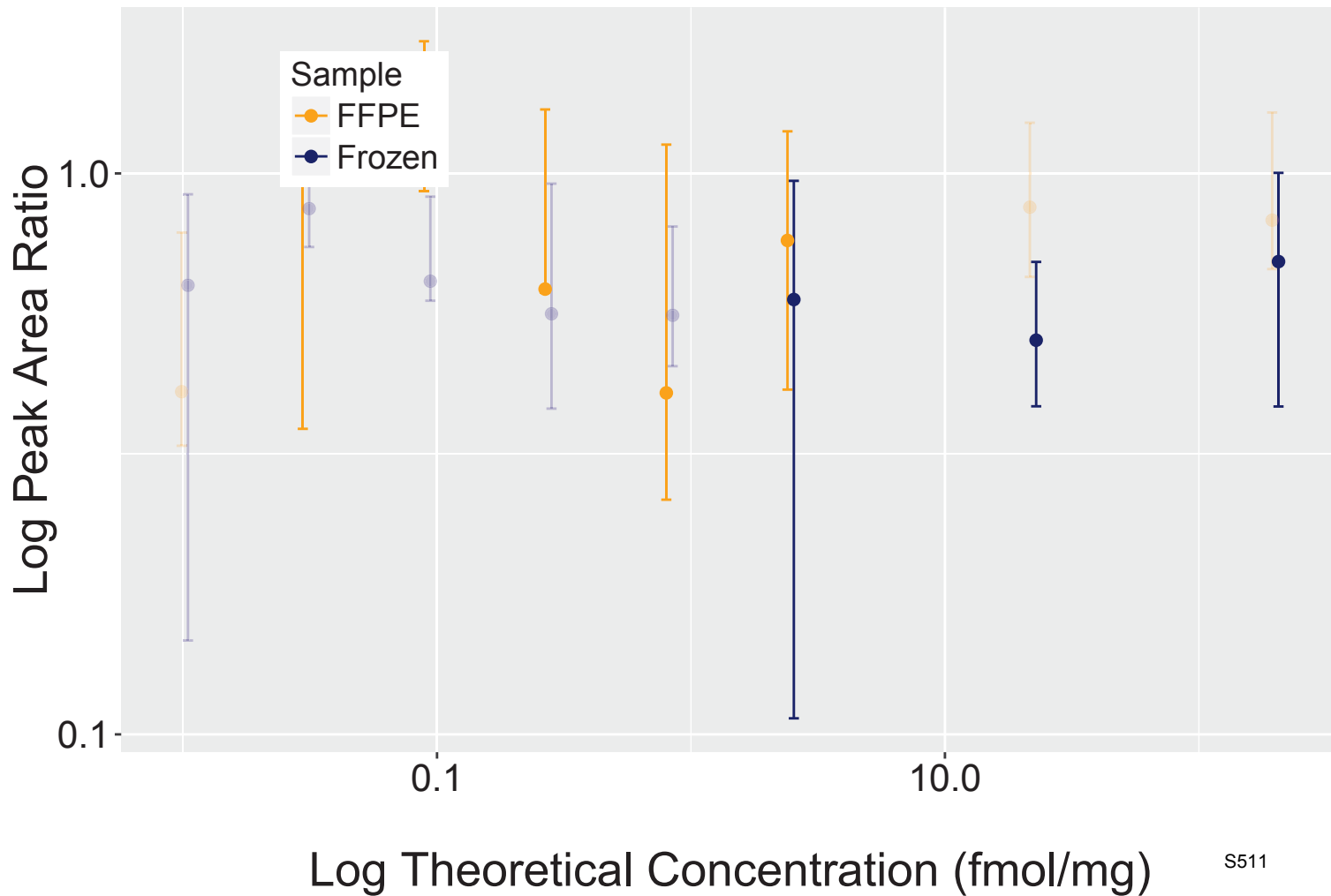
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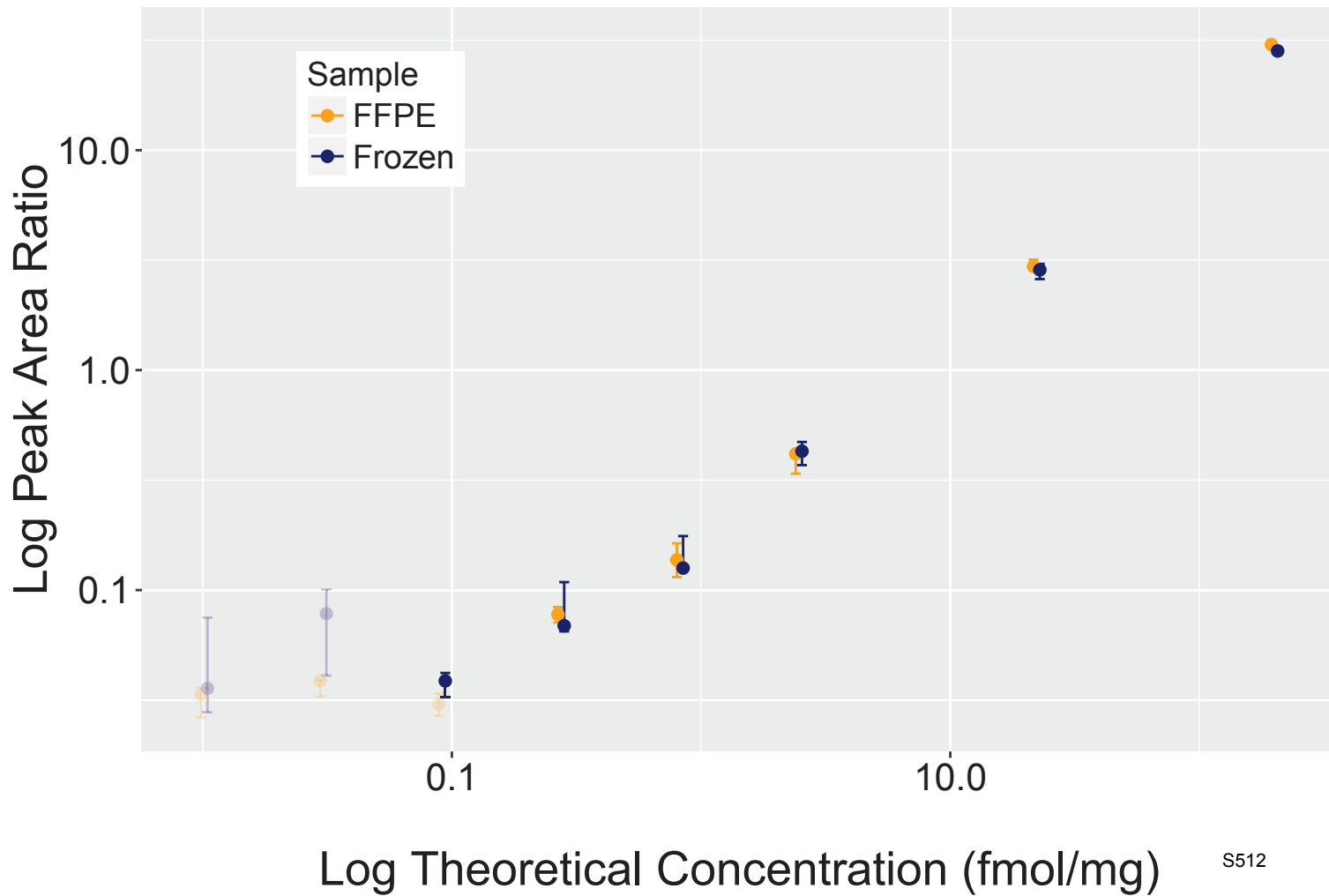
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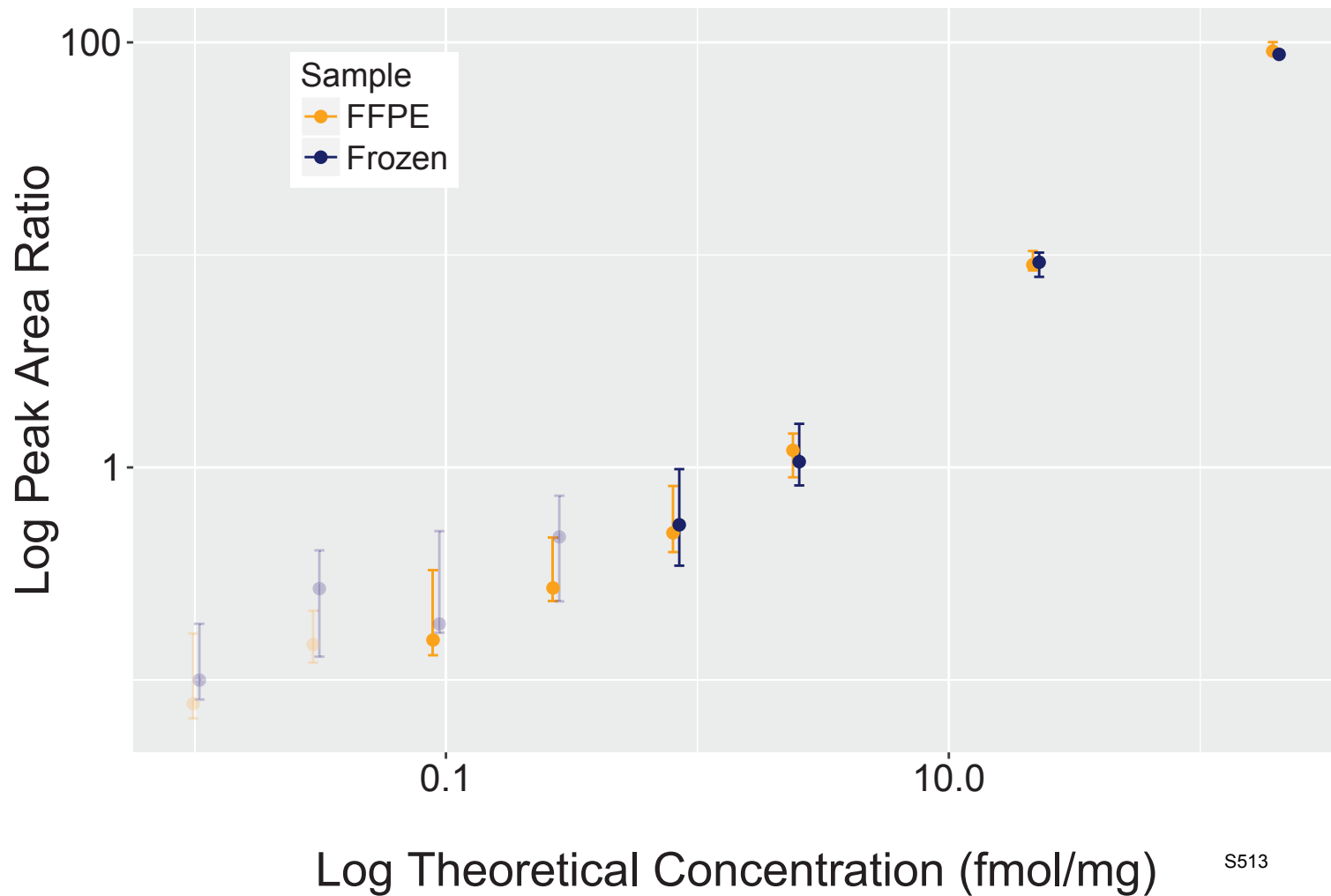
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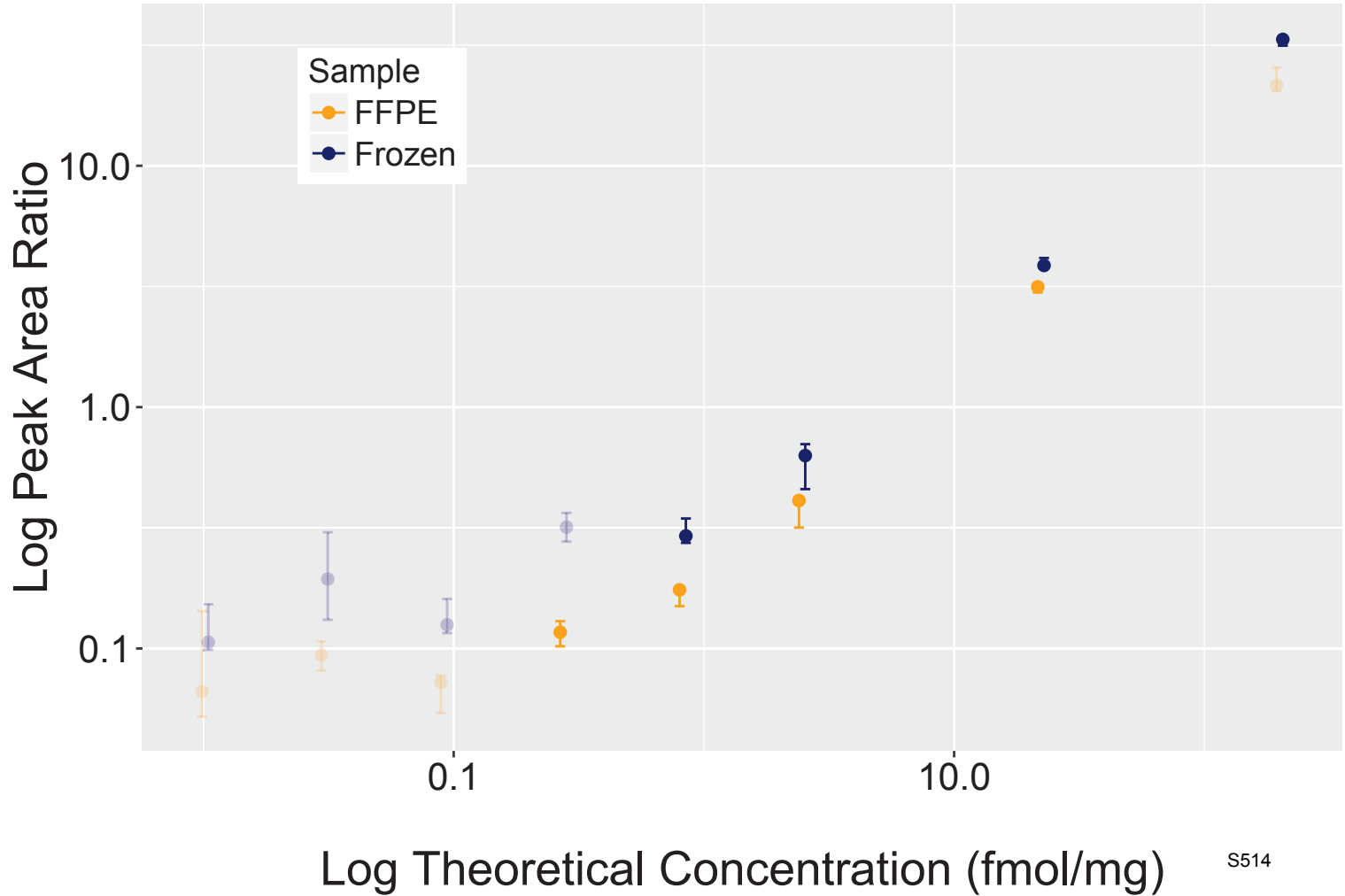
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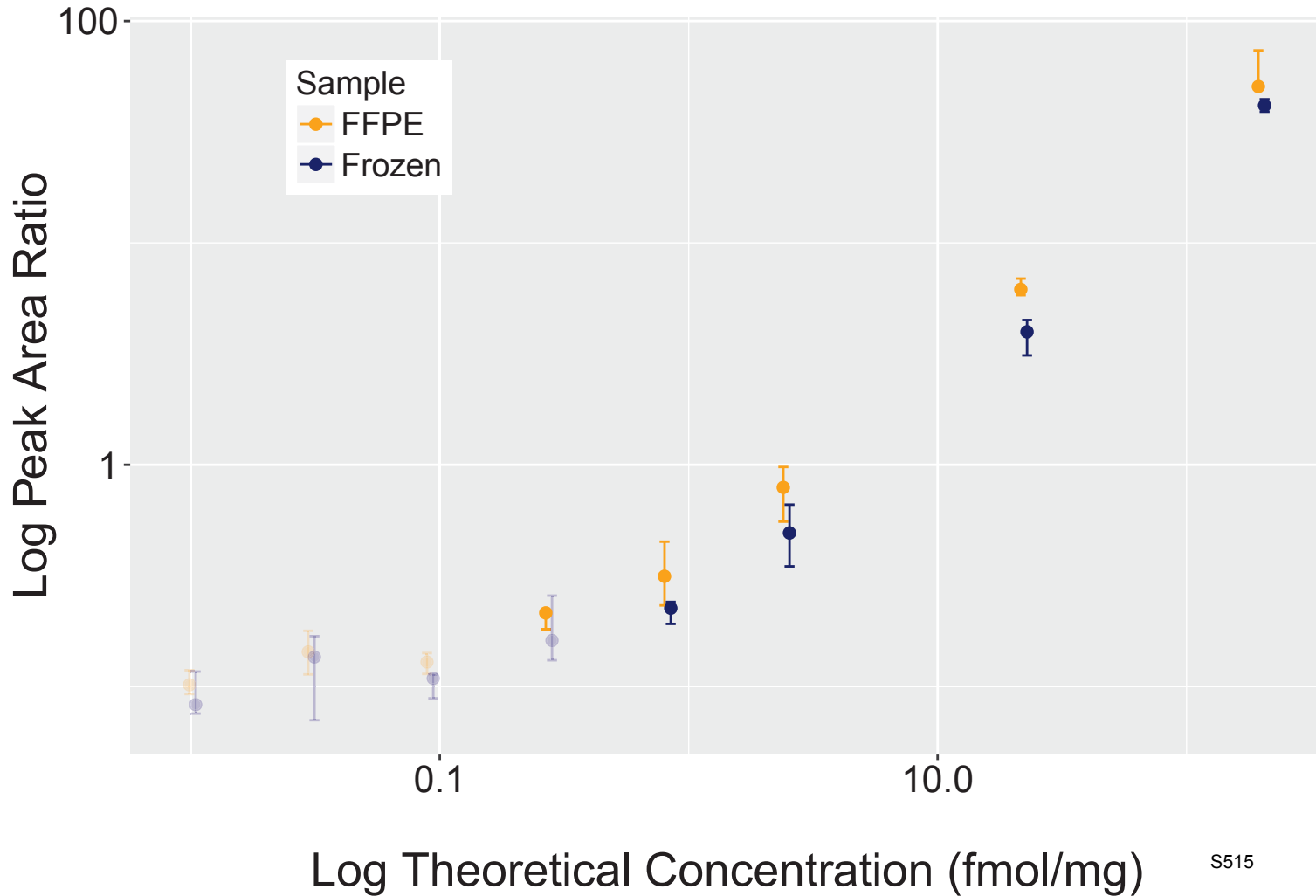
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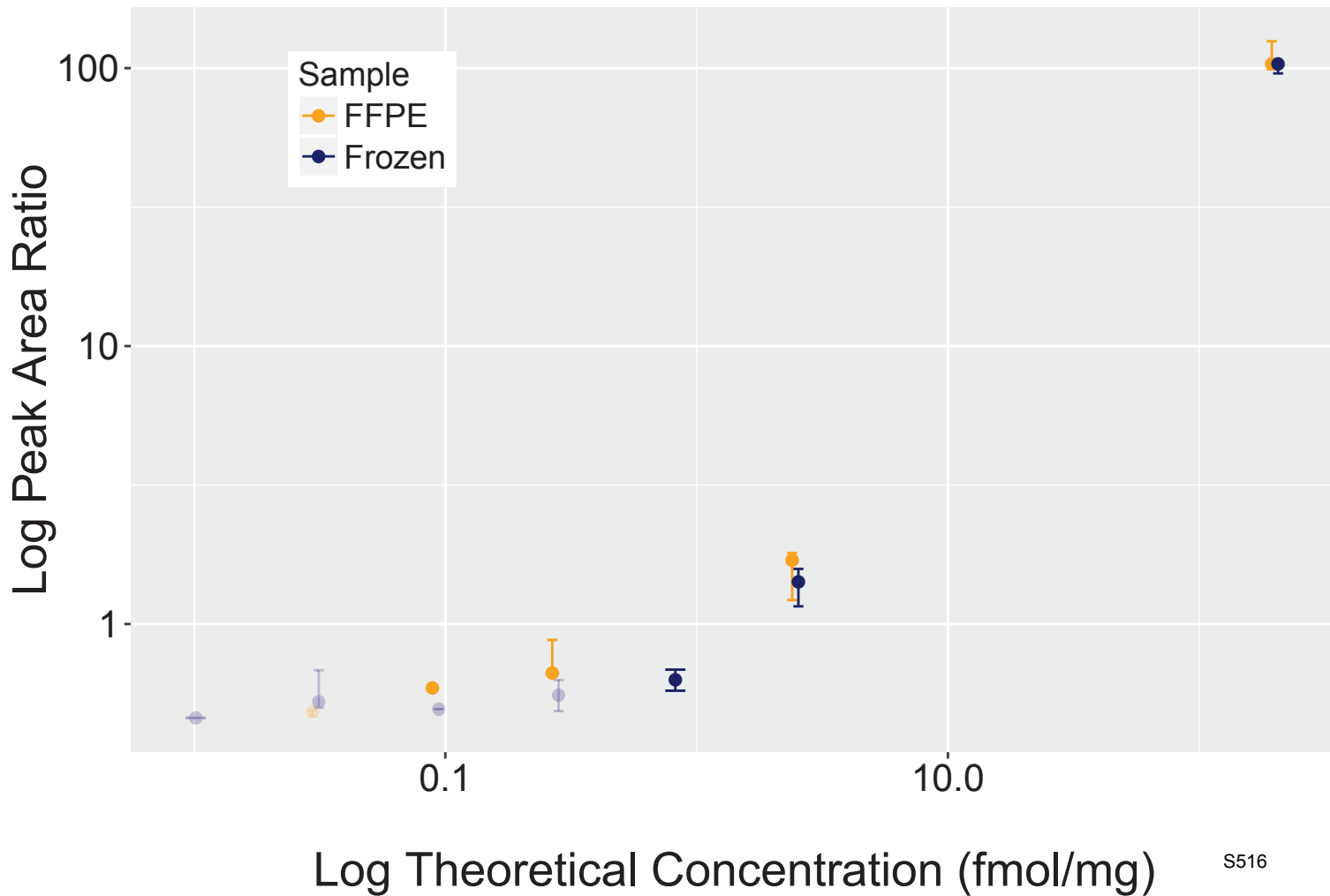
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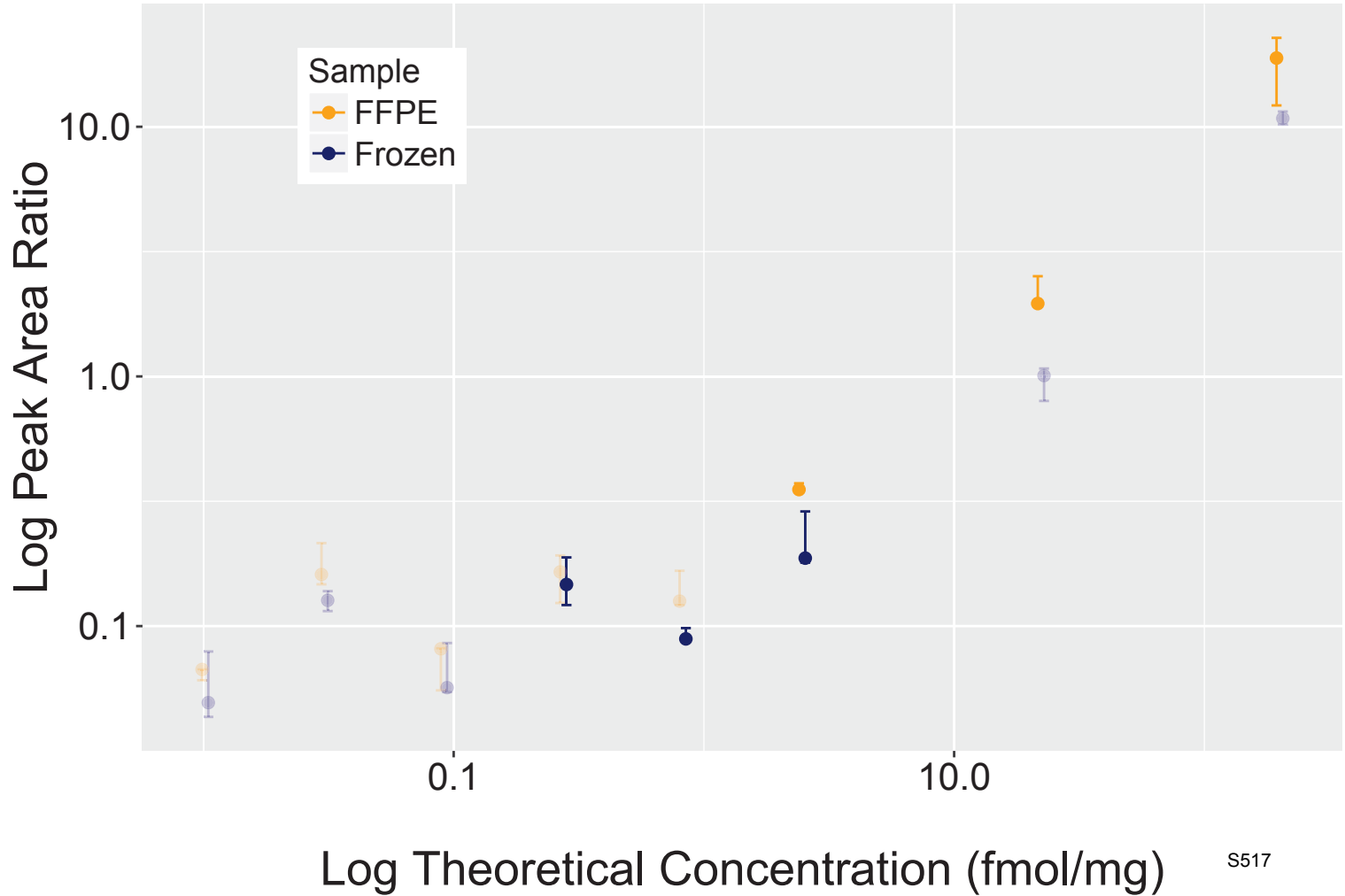
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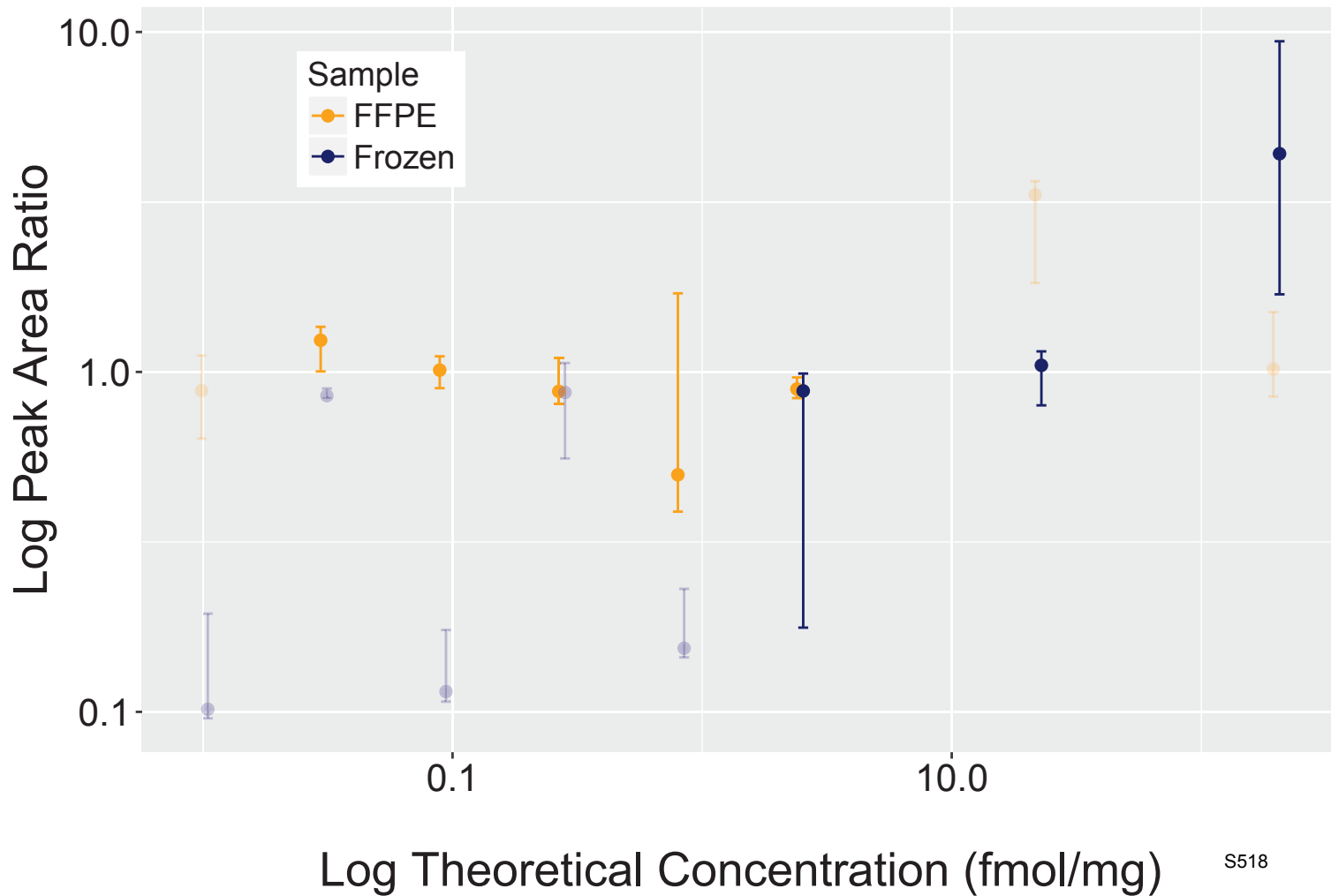
Analyte: MSH6.SVAPAAPTSC[+57]DFSPGDLVWAK



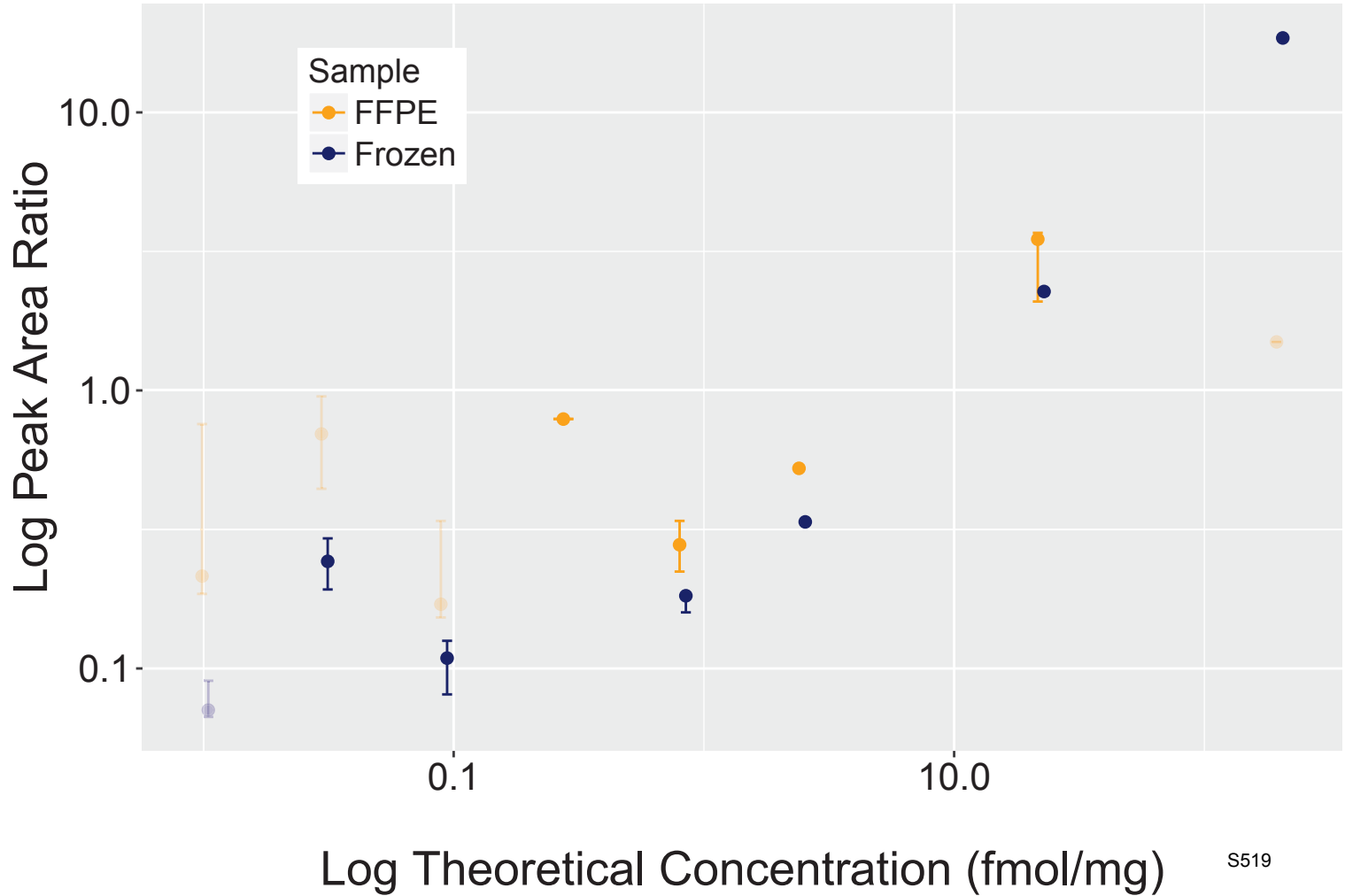
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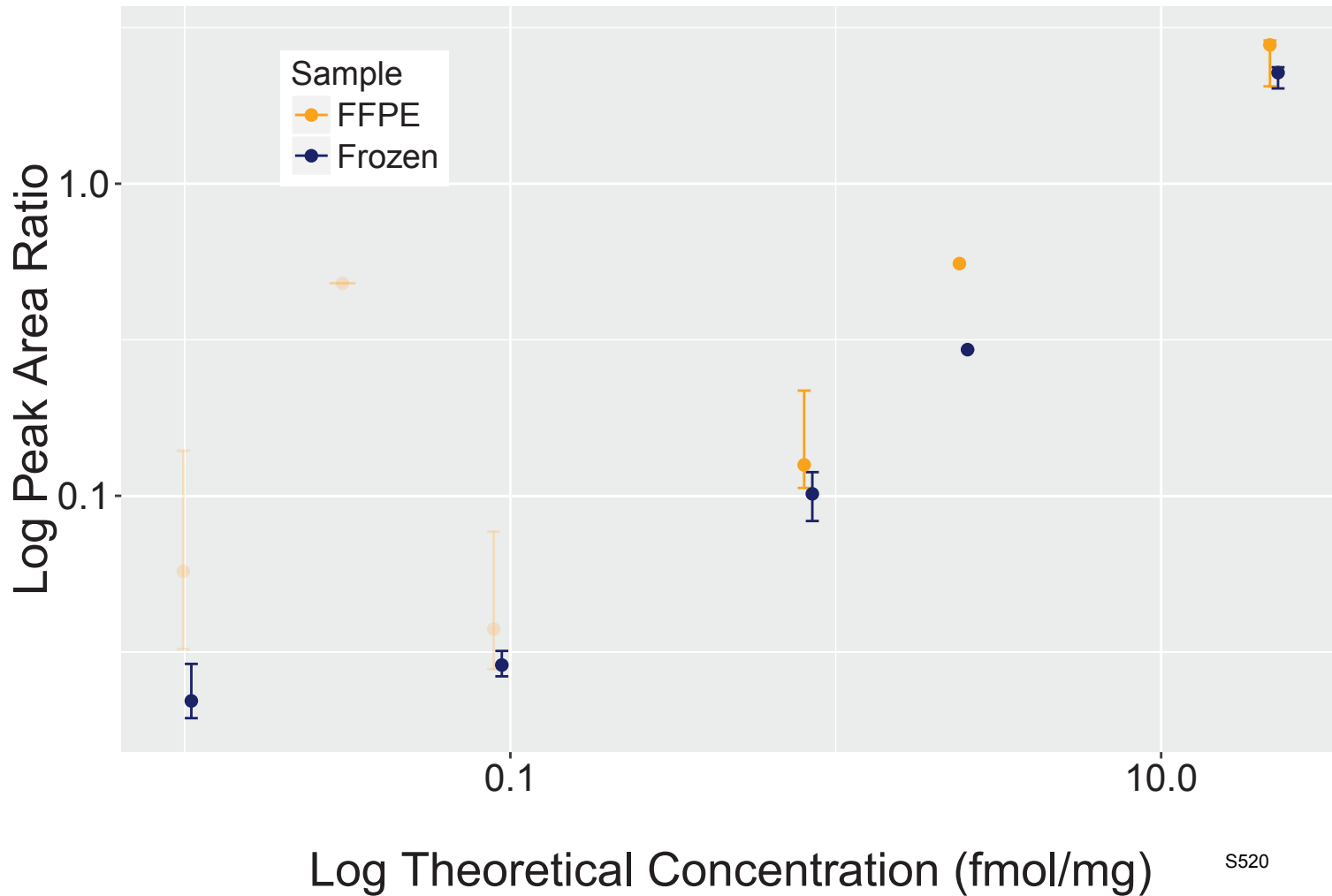
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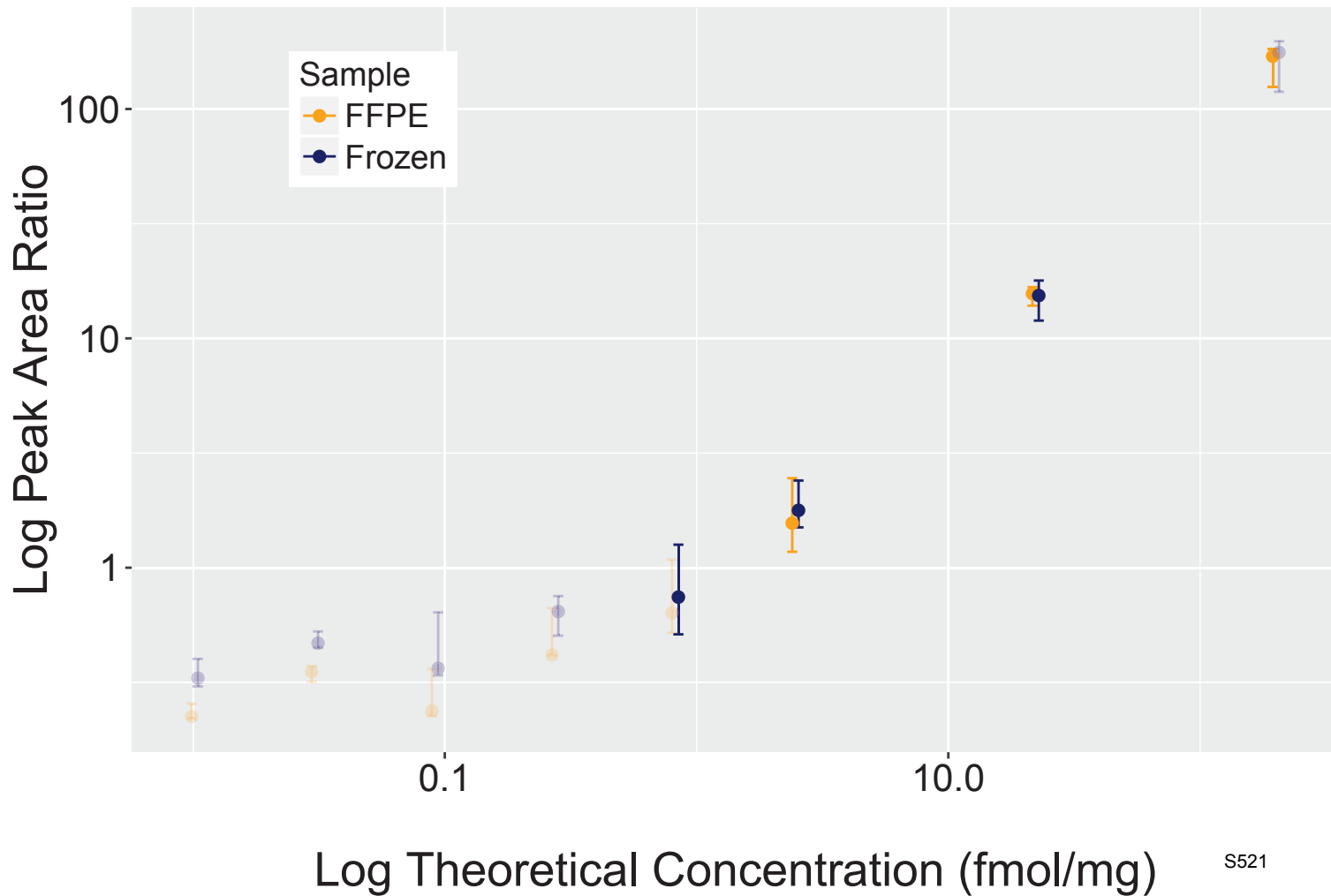
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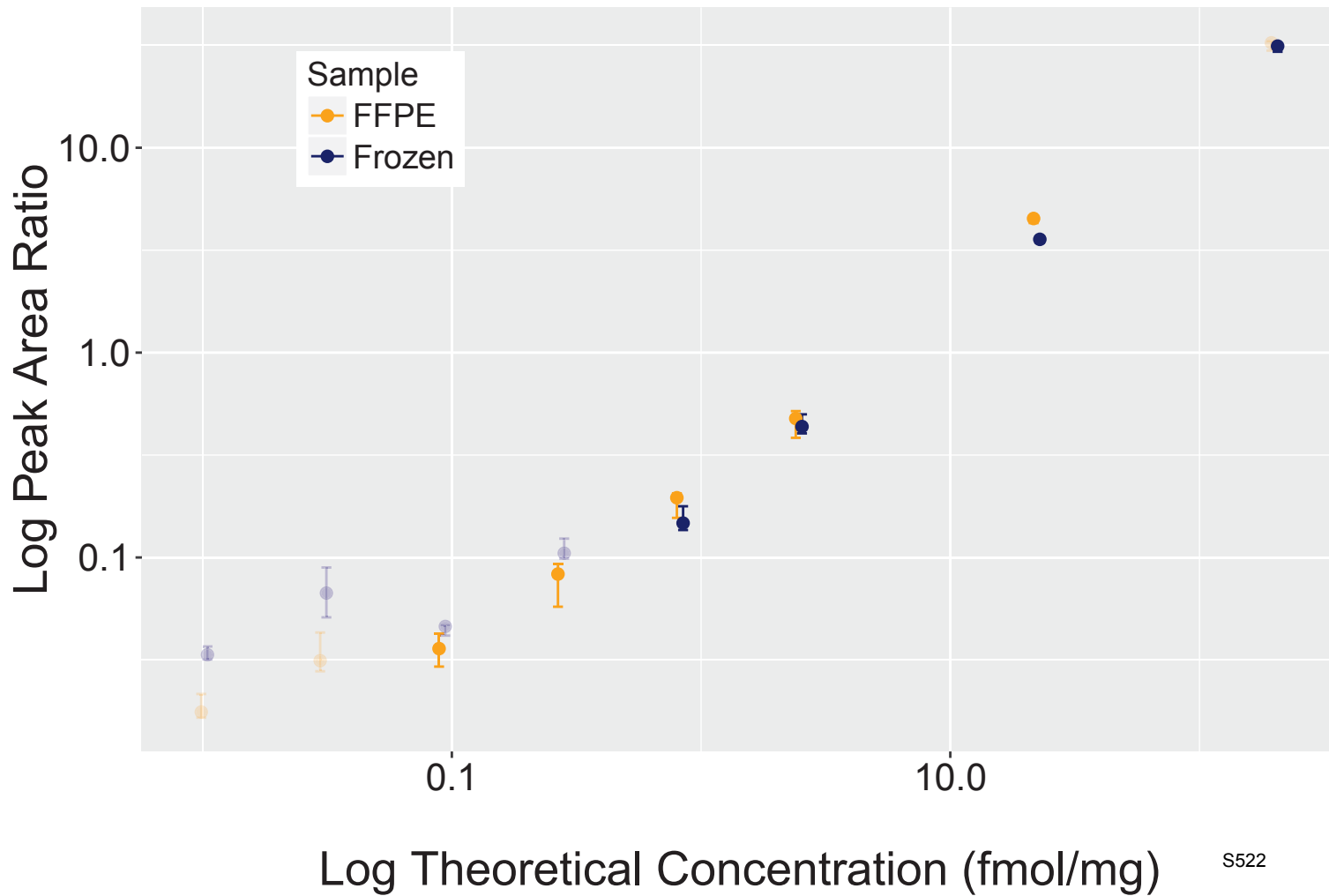
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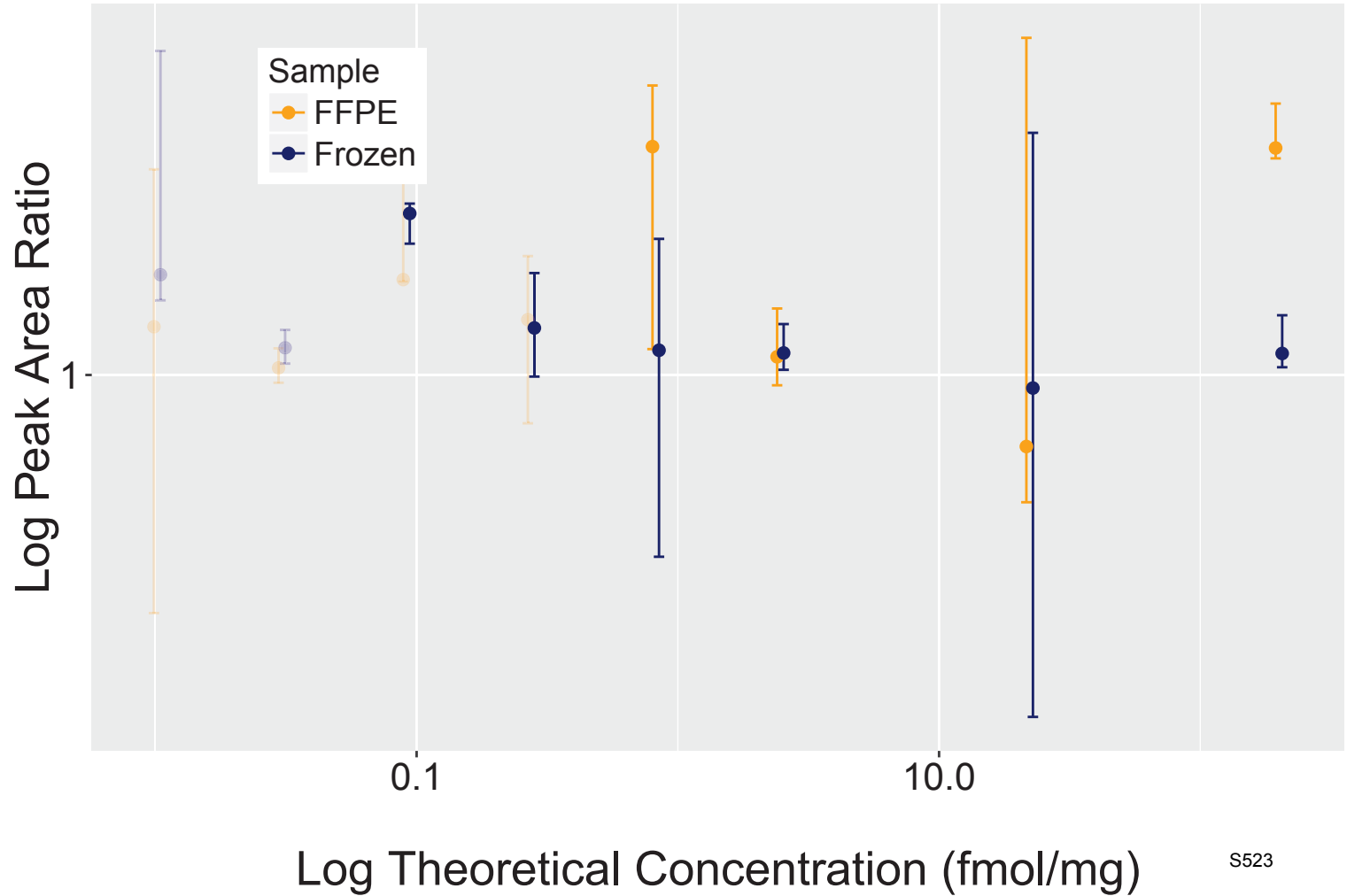
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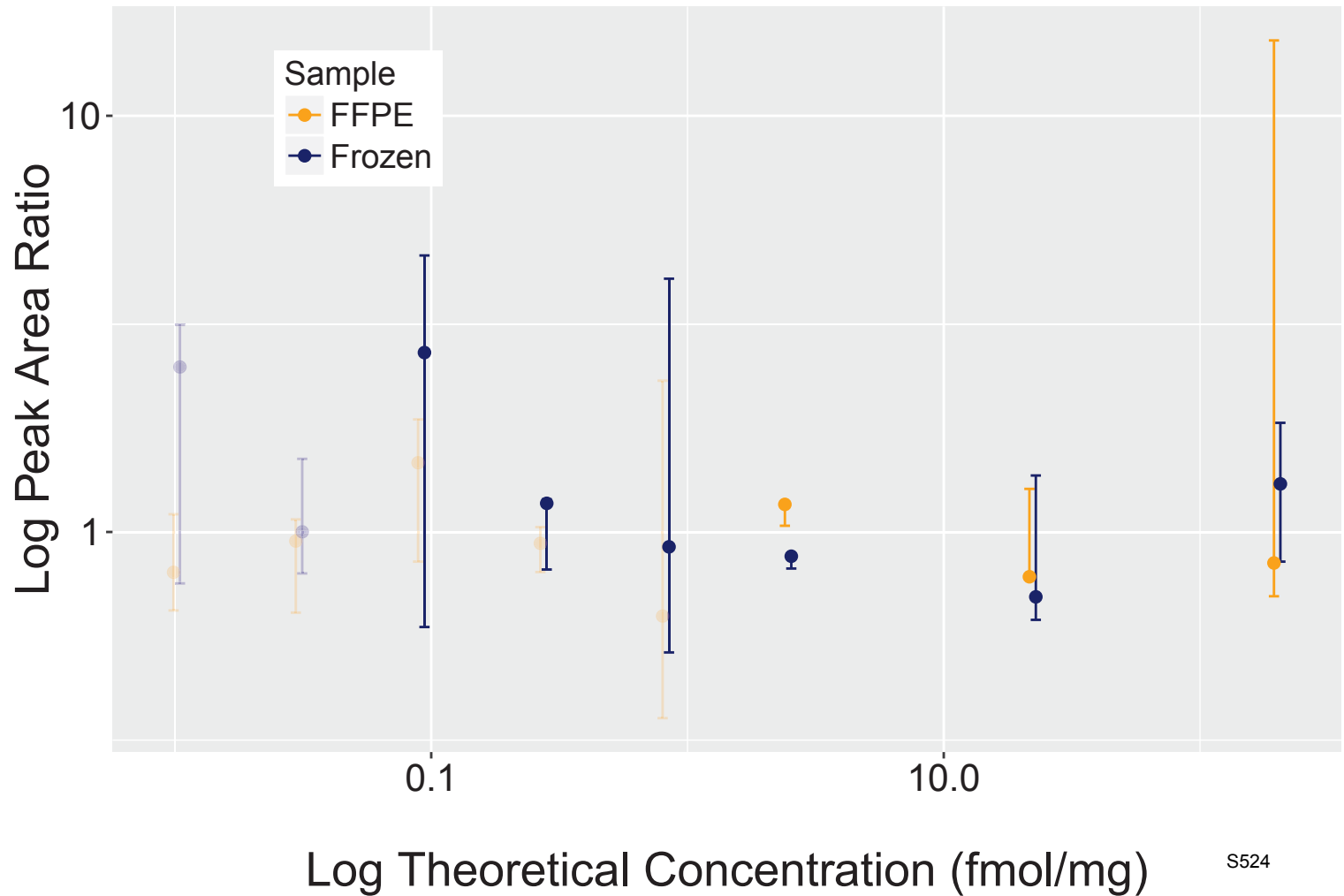
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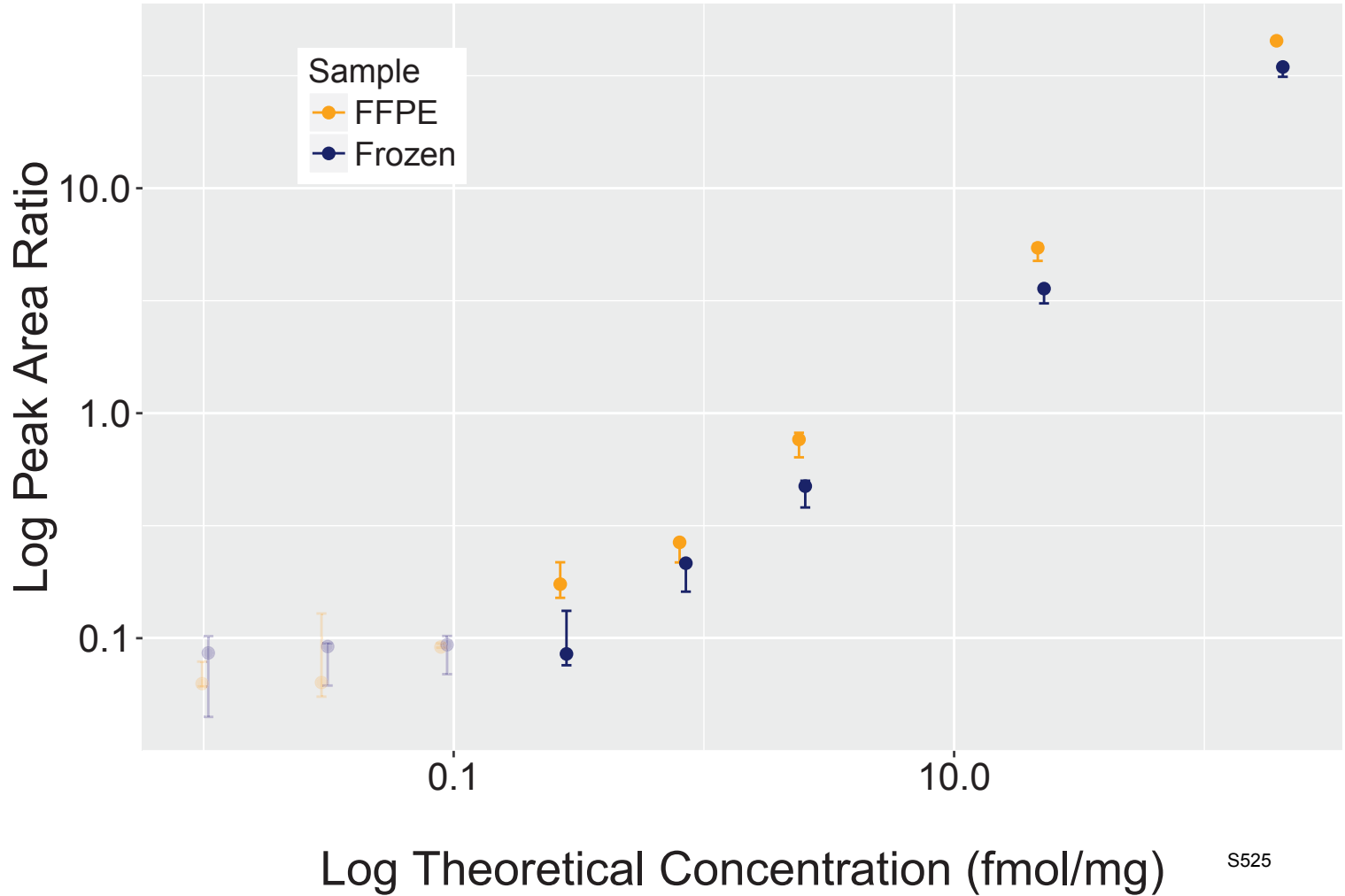
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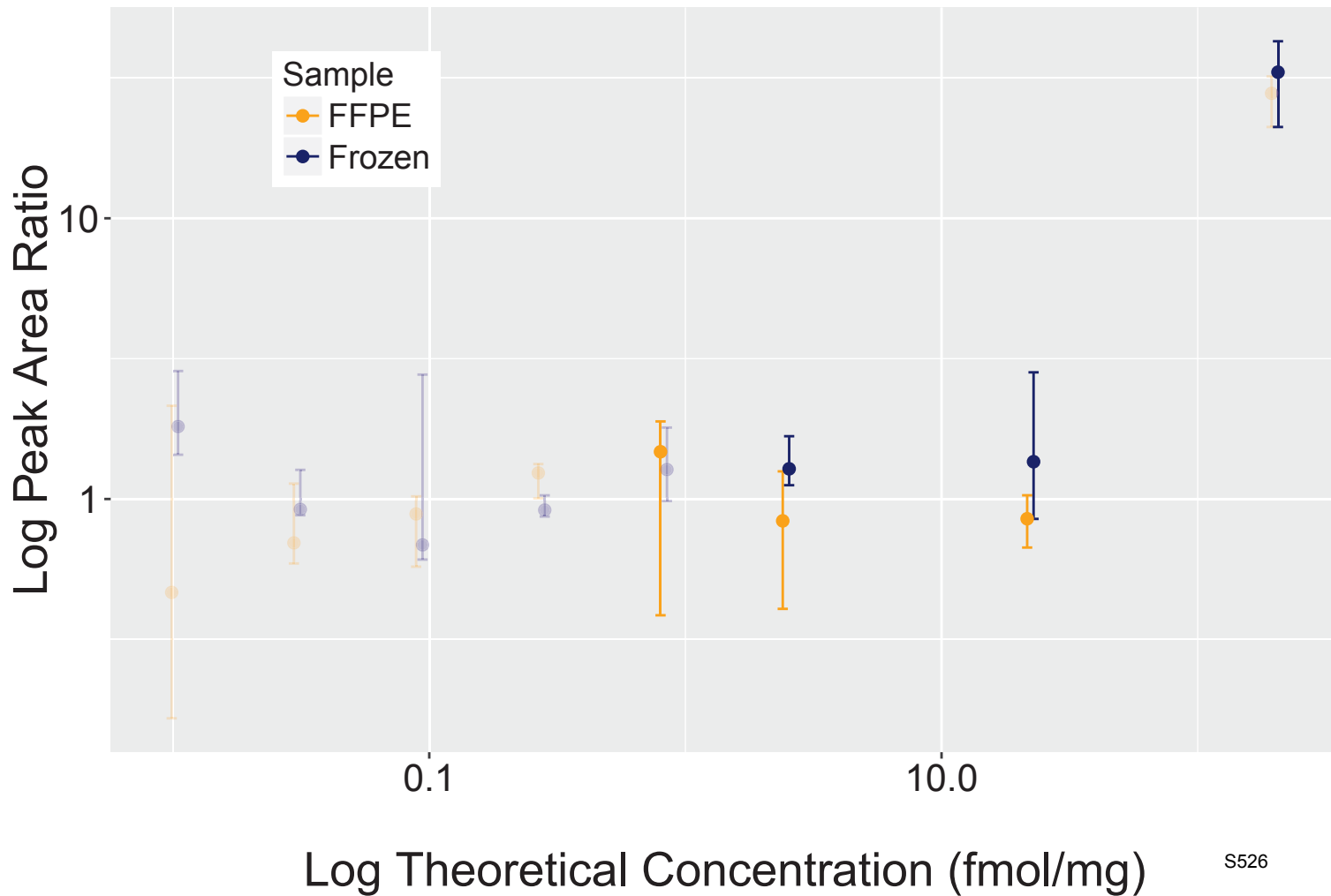
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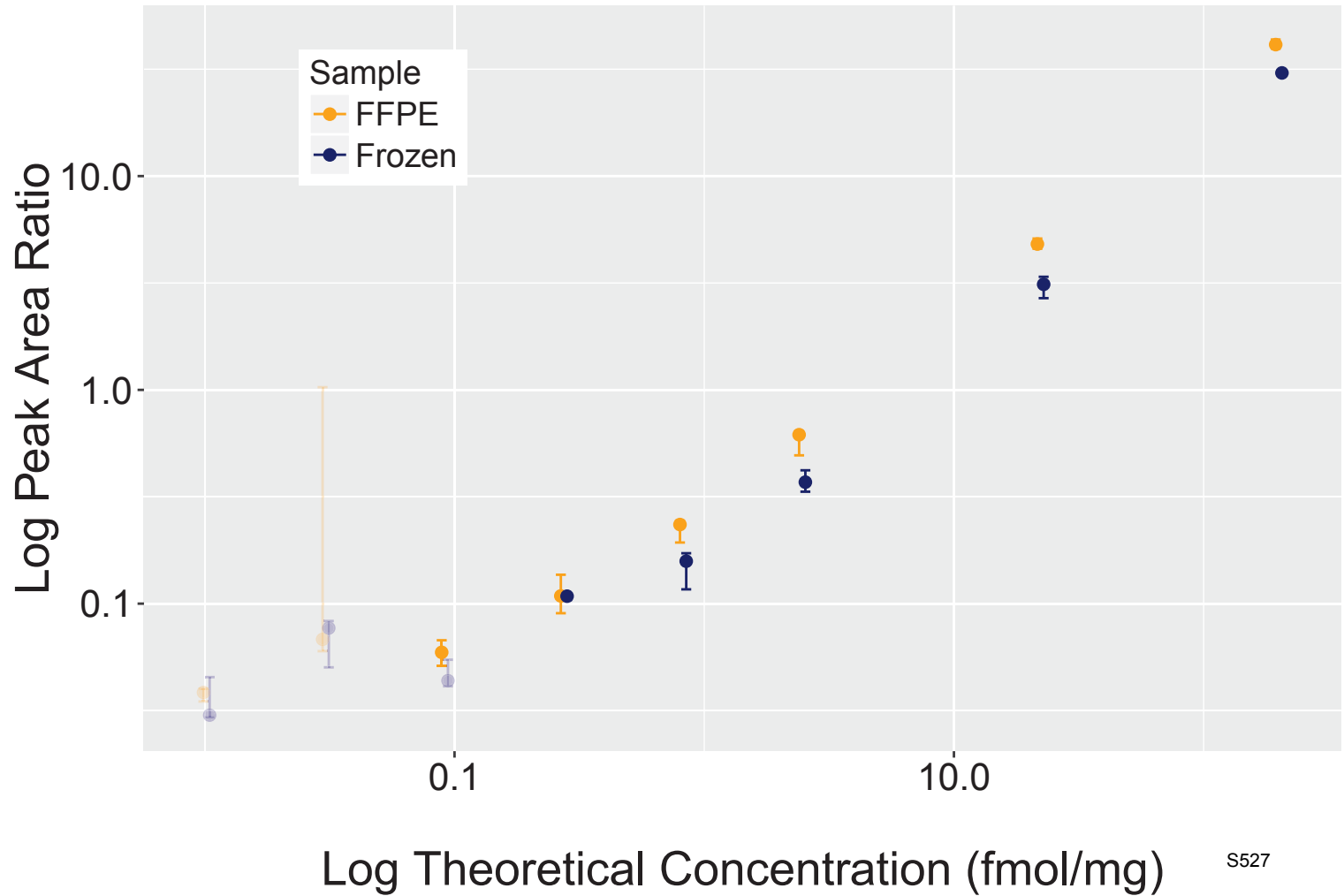
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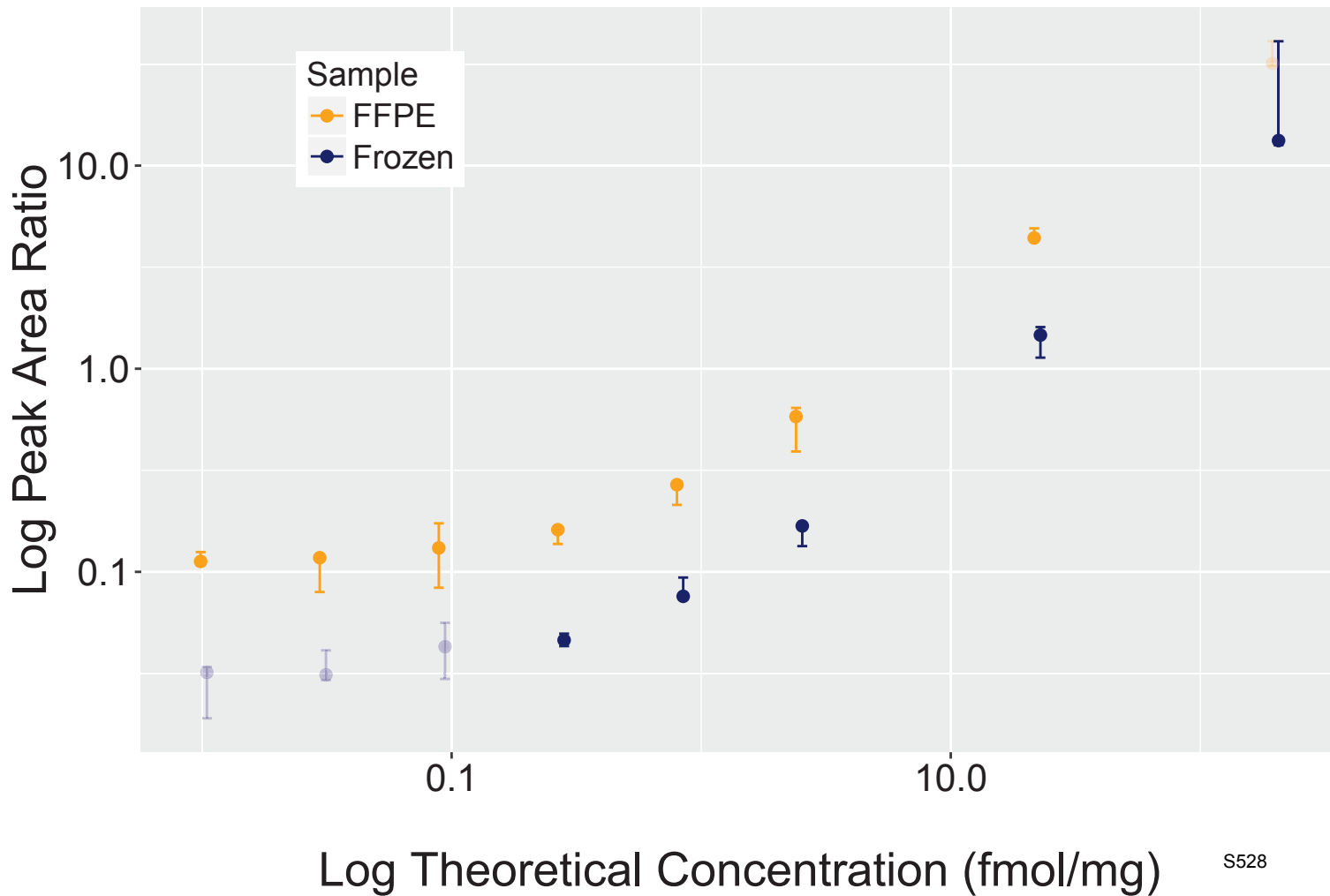
Analyte: UBAP2L.TATEEWGTEDWNEDLSETK



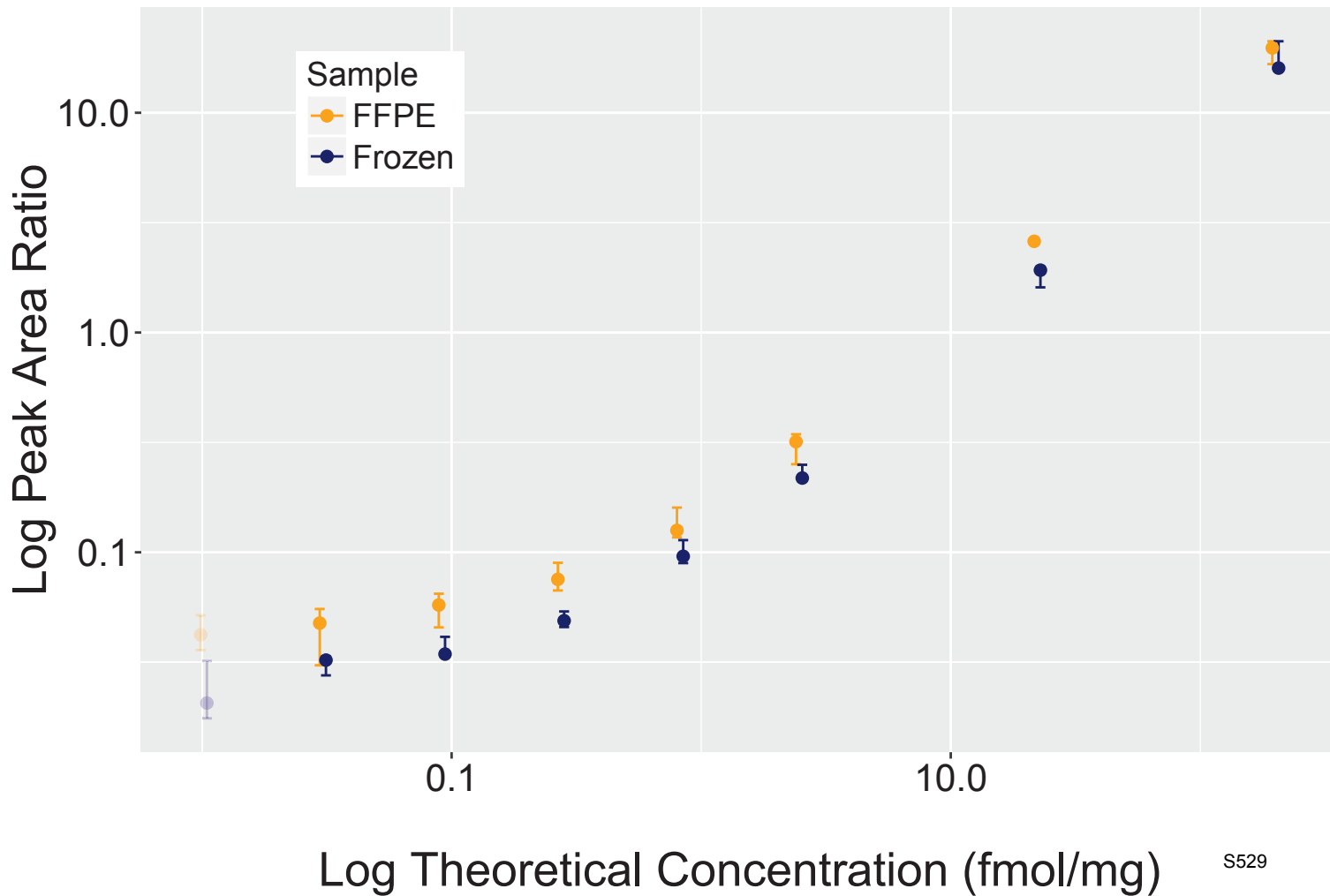
Analyte: CKAP4.TAVDSLVAYSVK



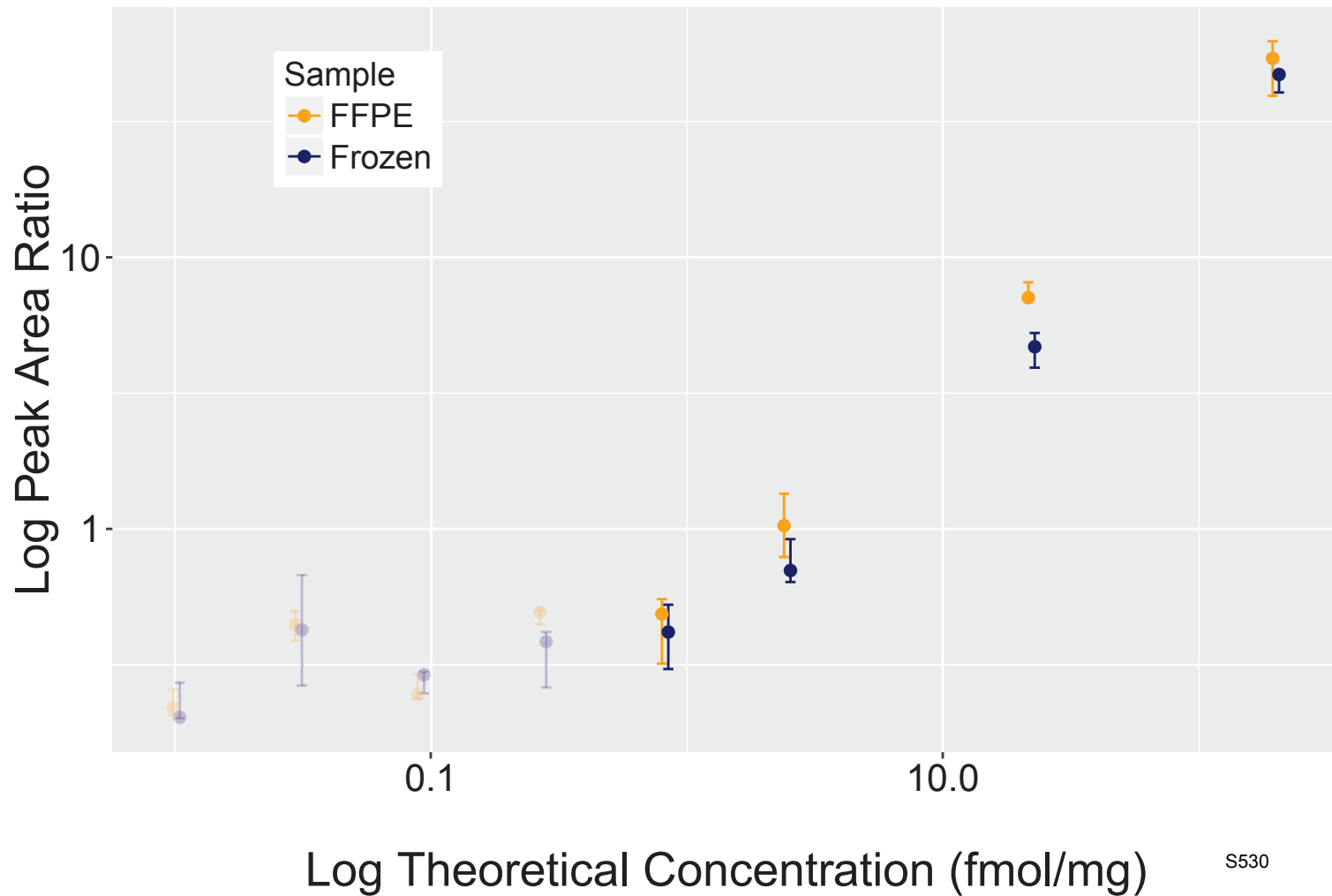
Analyte: GDI2.TDDYLDQPC[+57]YETINR



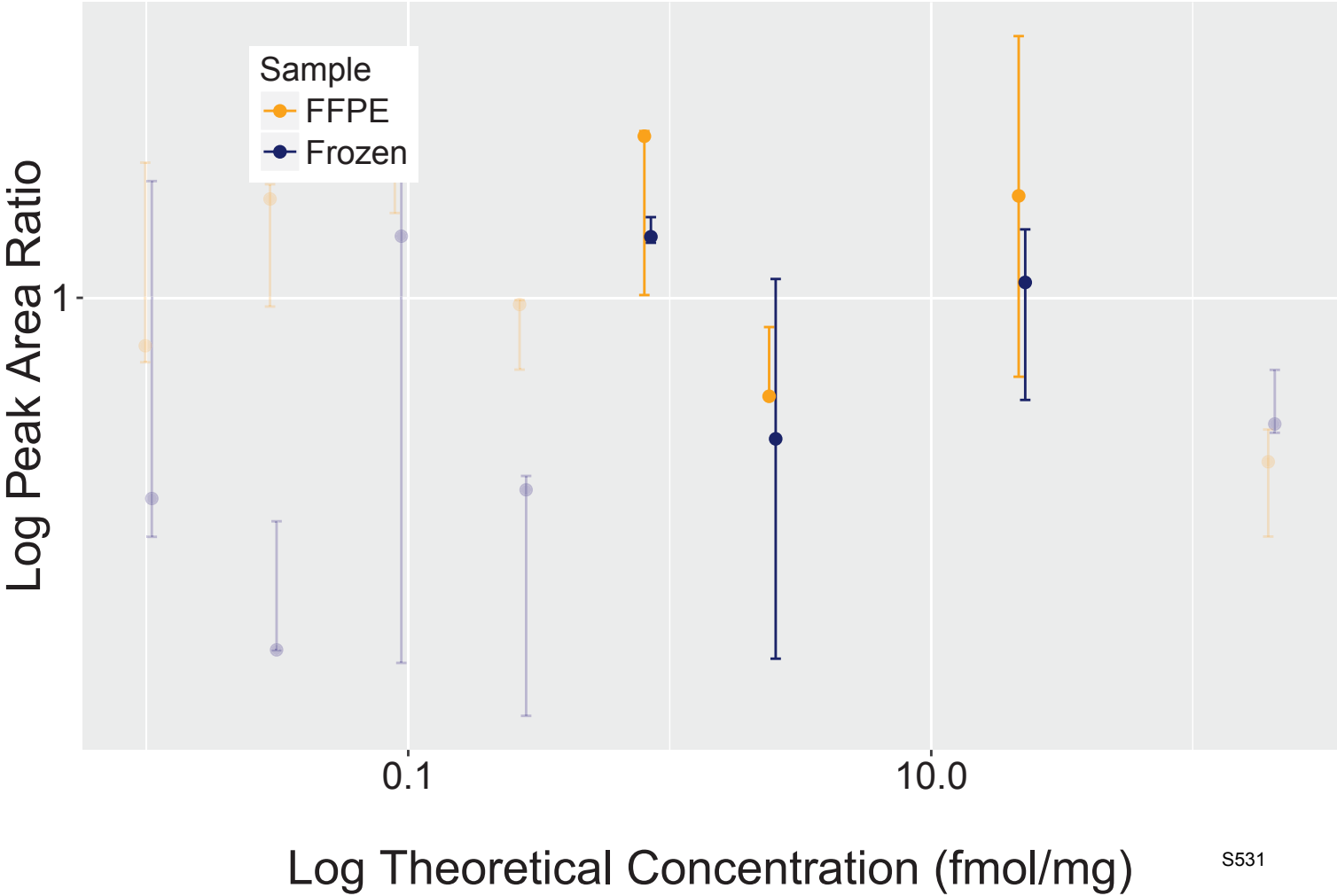
Analyte: CAPNS1.TDGFGIDTC[+57]R



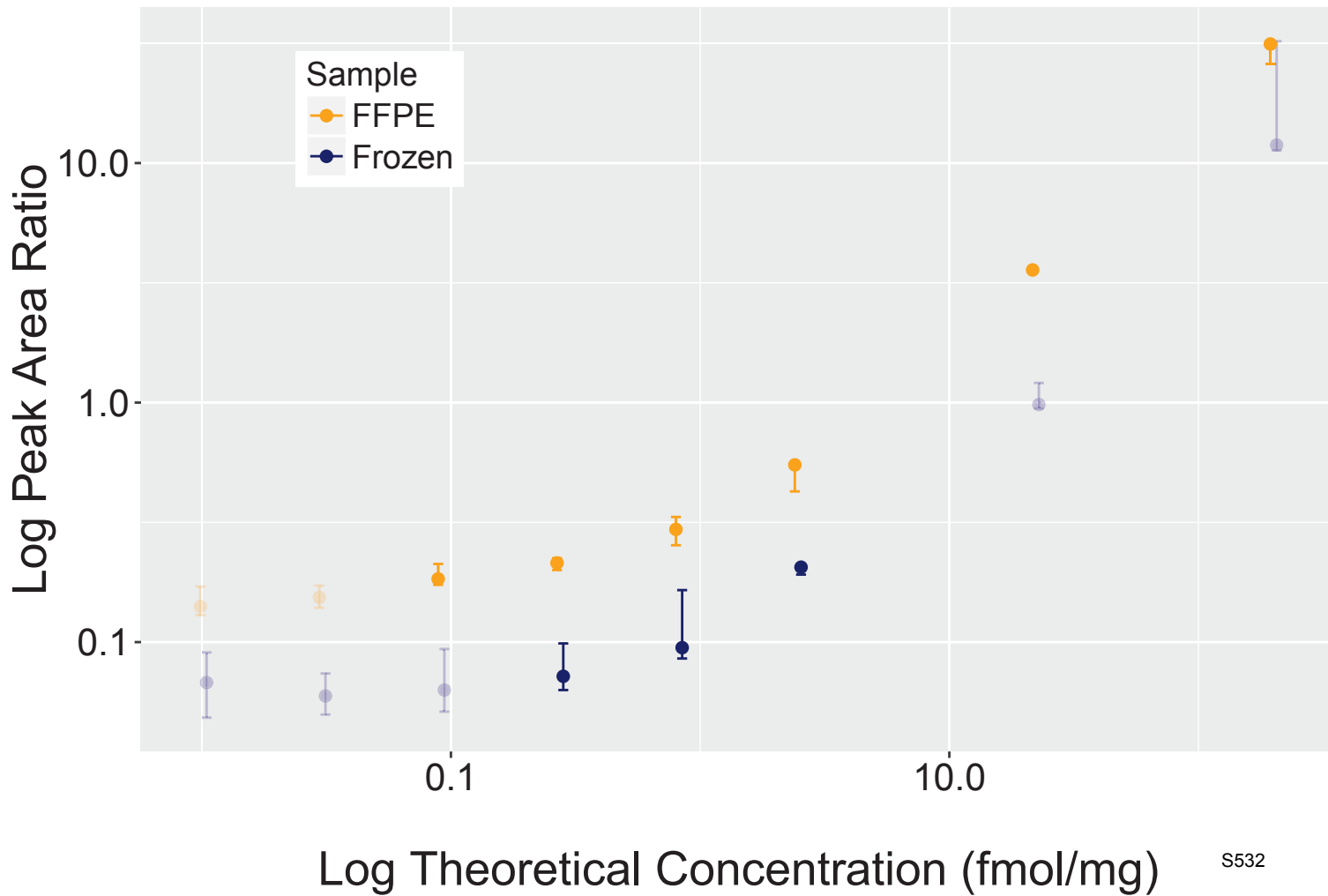
Analyte: DUT.TDIQIALPSGC[+57]YGR



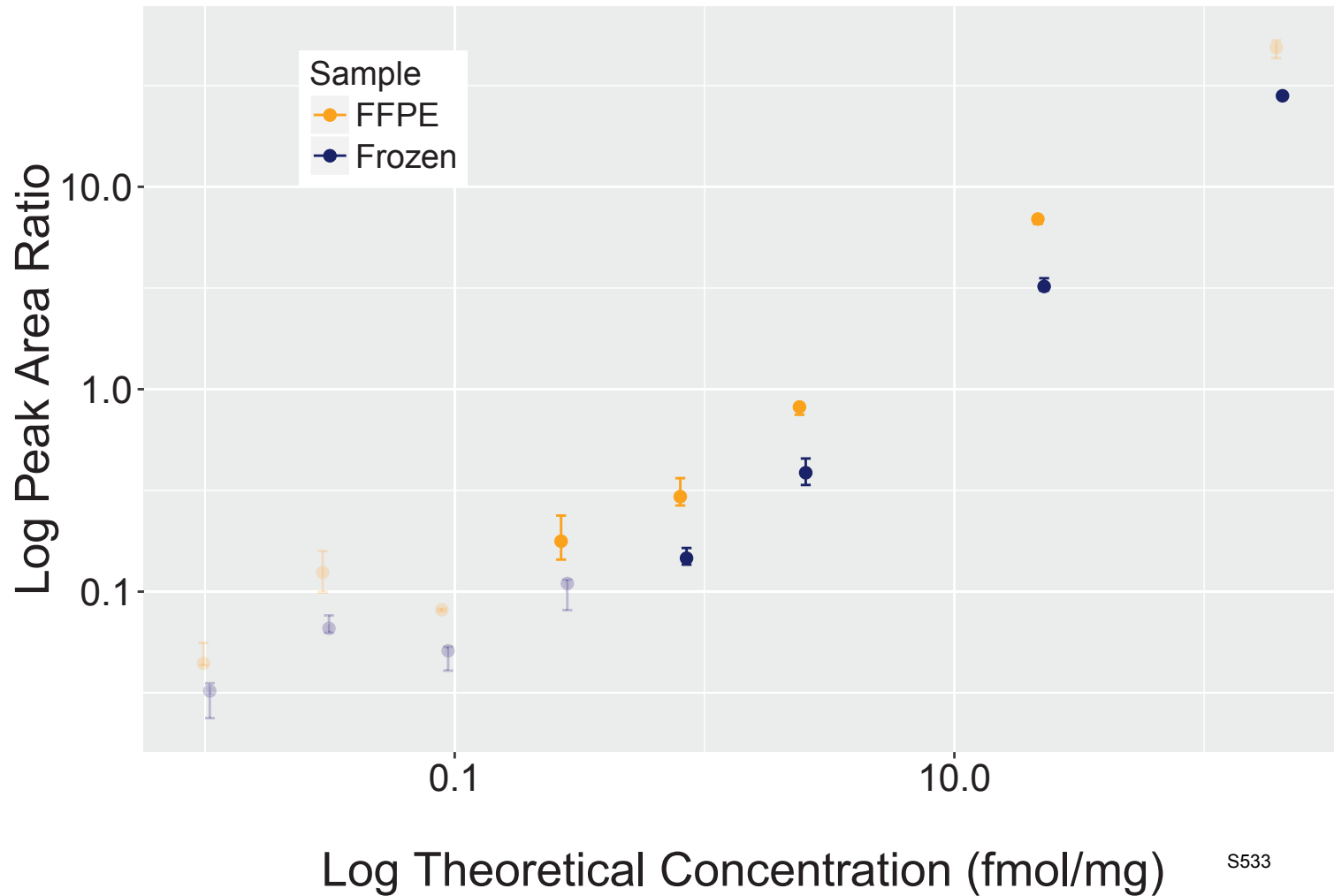
Analyte: ALDH18A1.TDLLIVLSDVEGLFDSPPGSDDAK



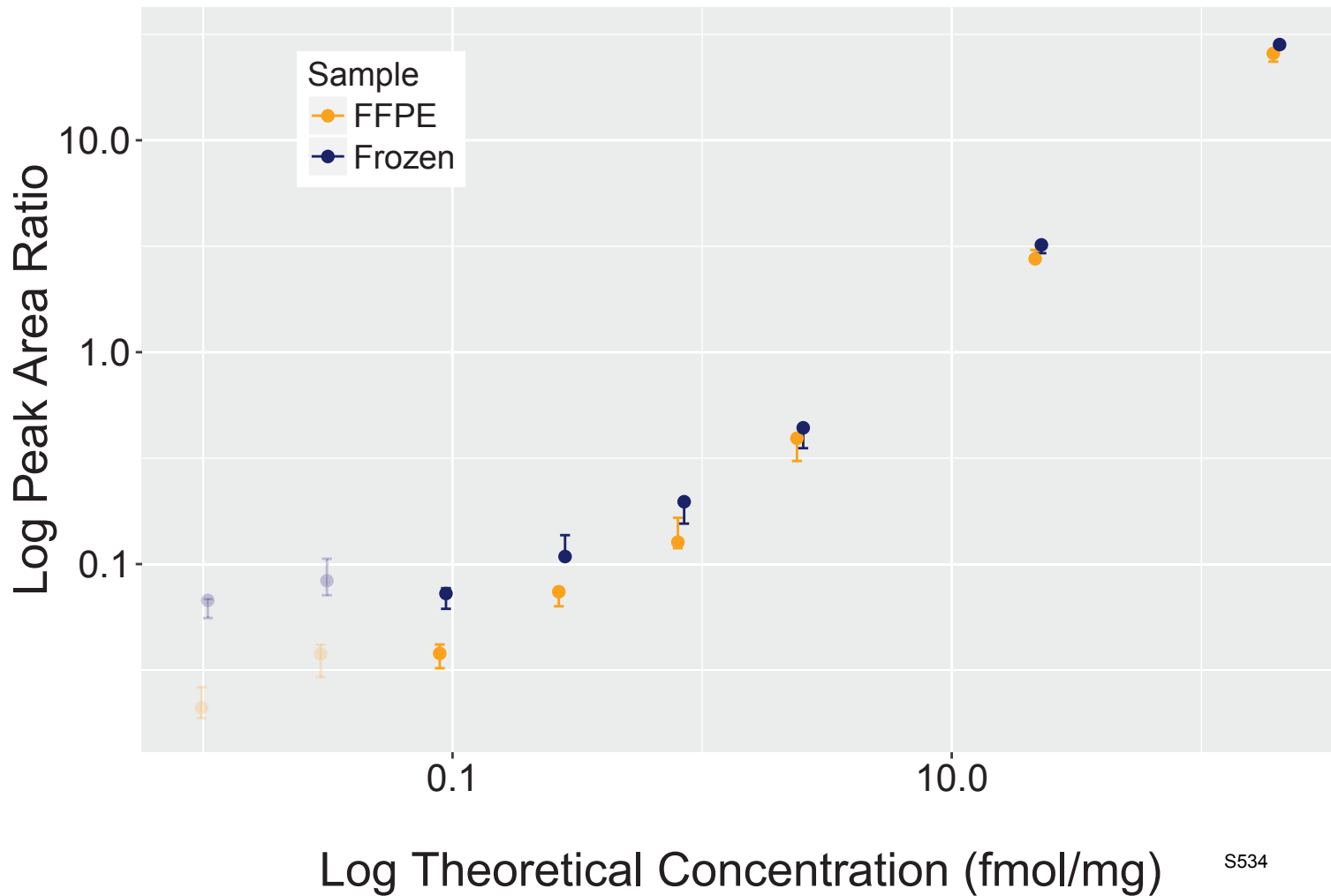
Analyte: XRCC5.TDTLEDLFP TTK



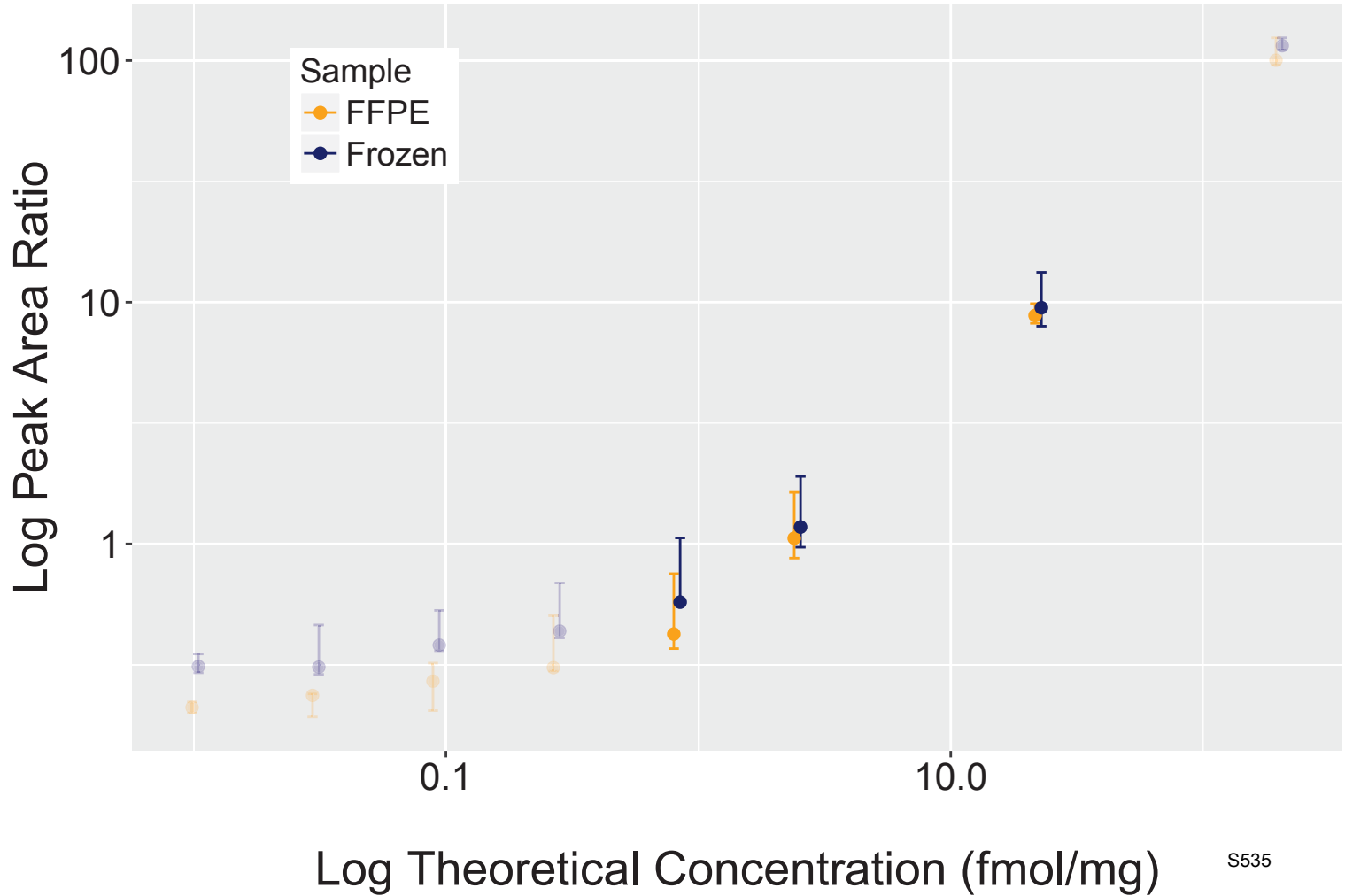
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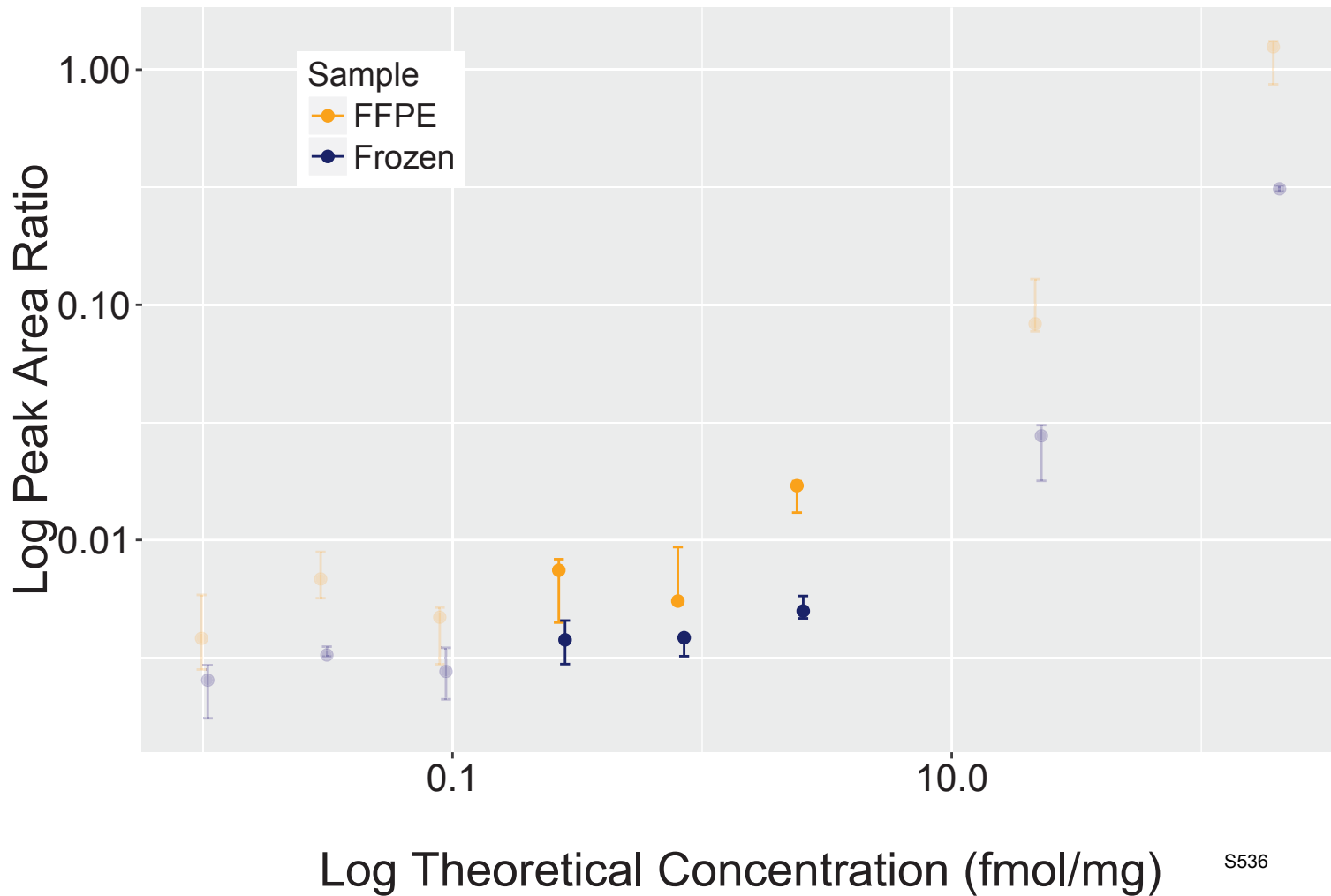
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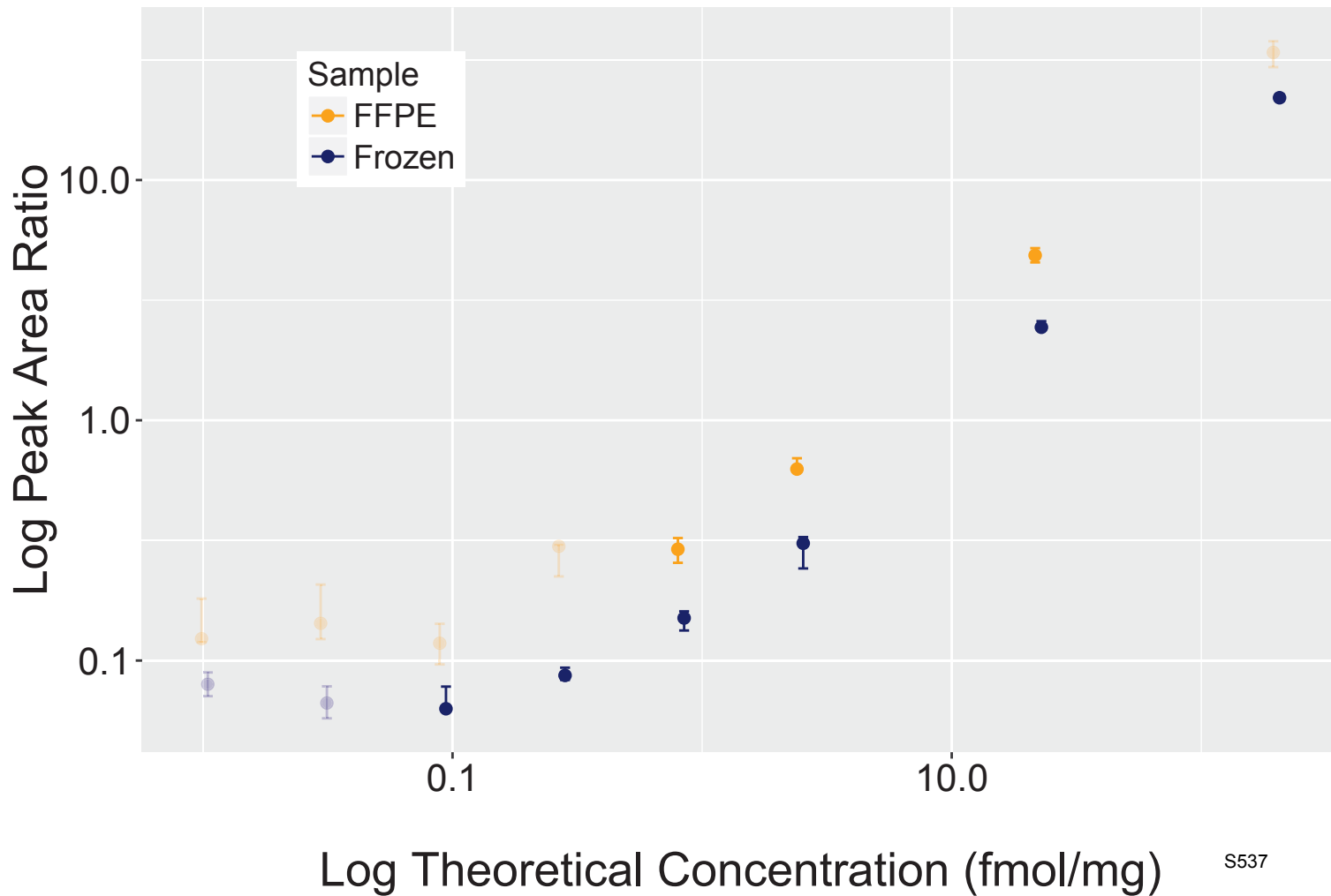
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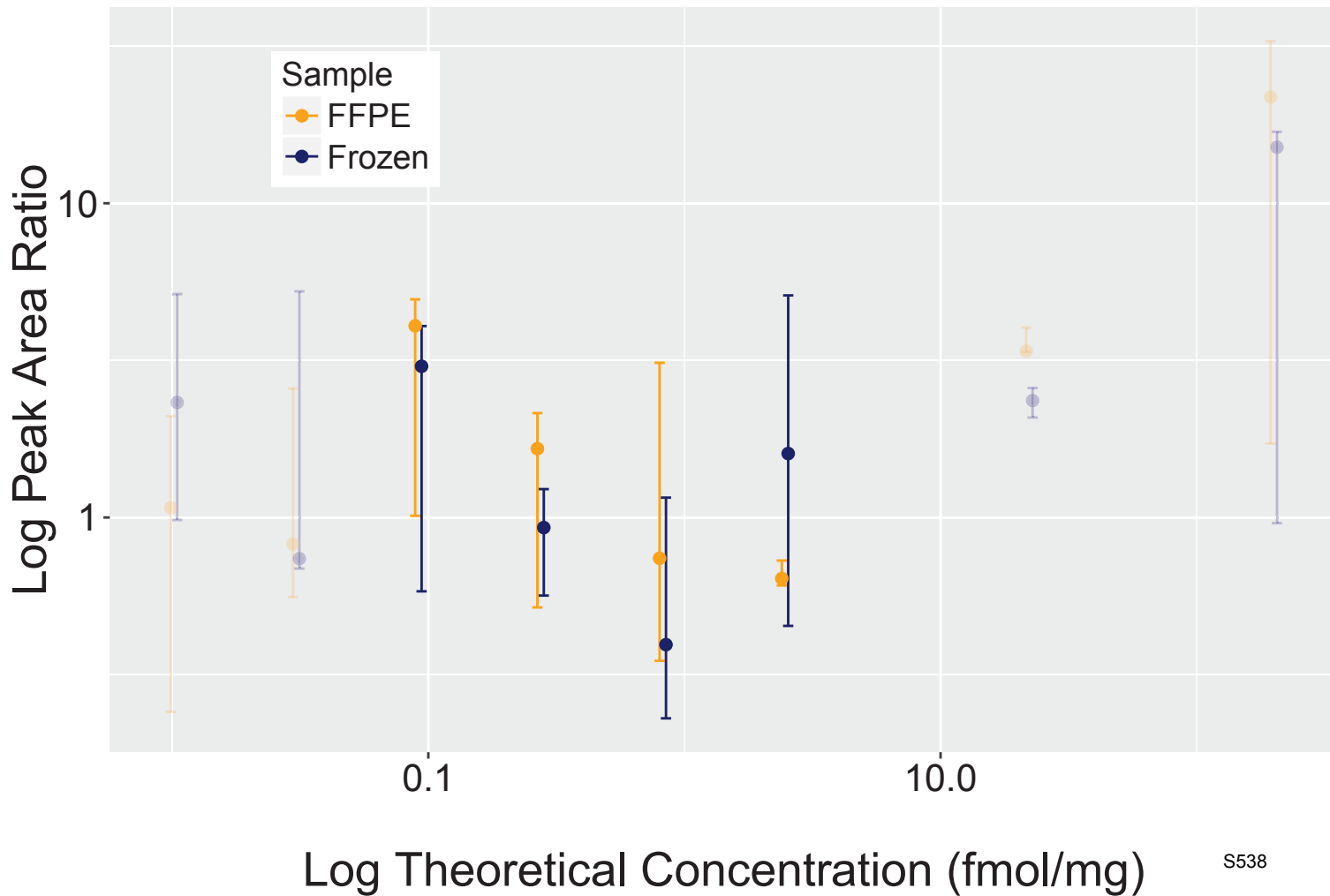
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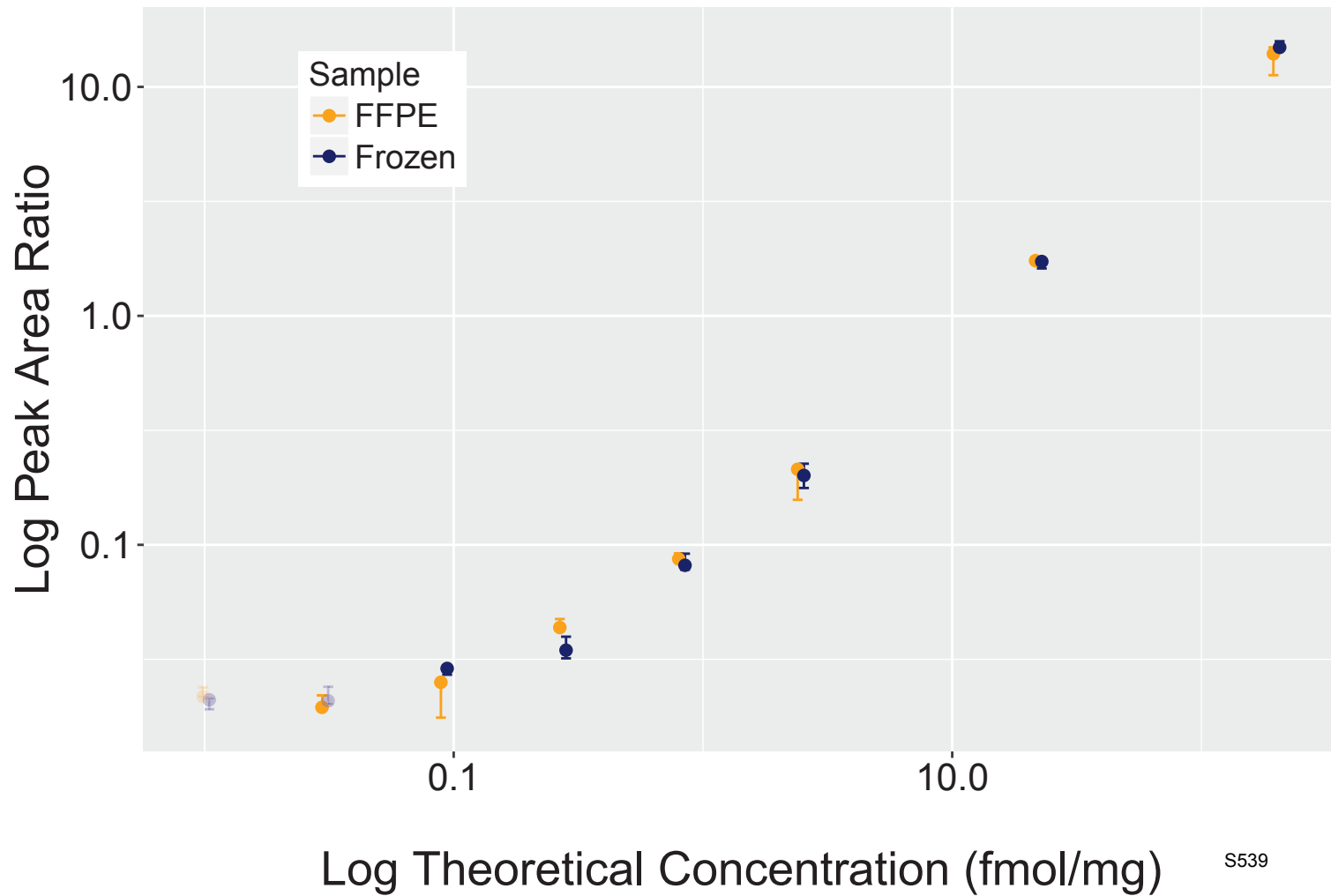
Analyte: HADHA.TGIEQGSDAGYLC[+57]ESQK



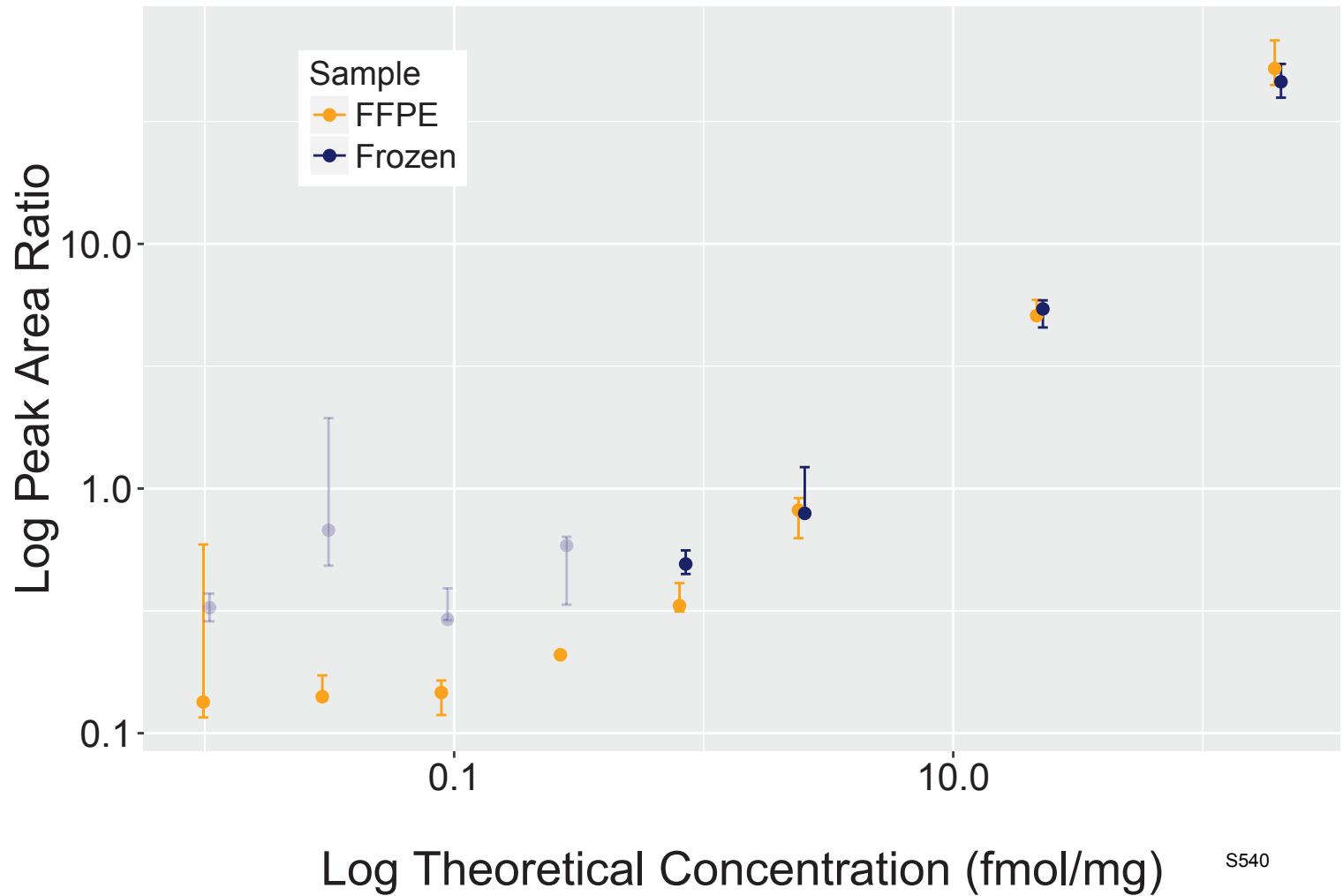
Analyte: PPP2R4.TGPFAEHSNQLWNISAVPSWSK



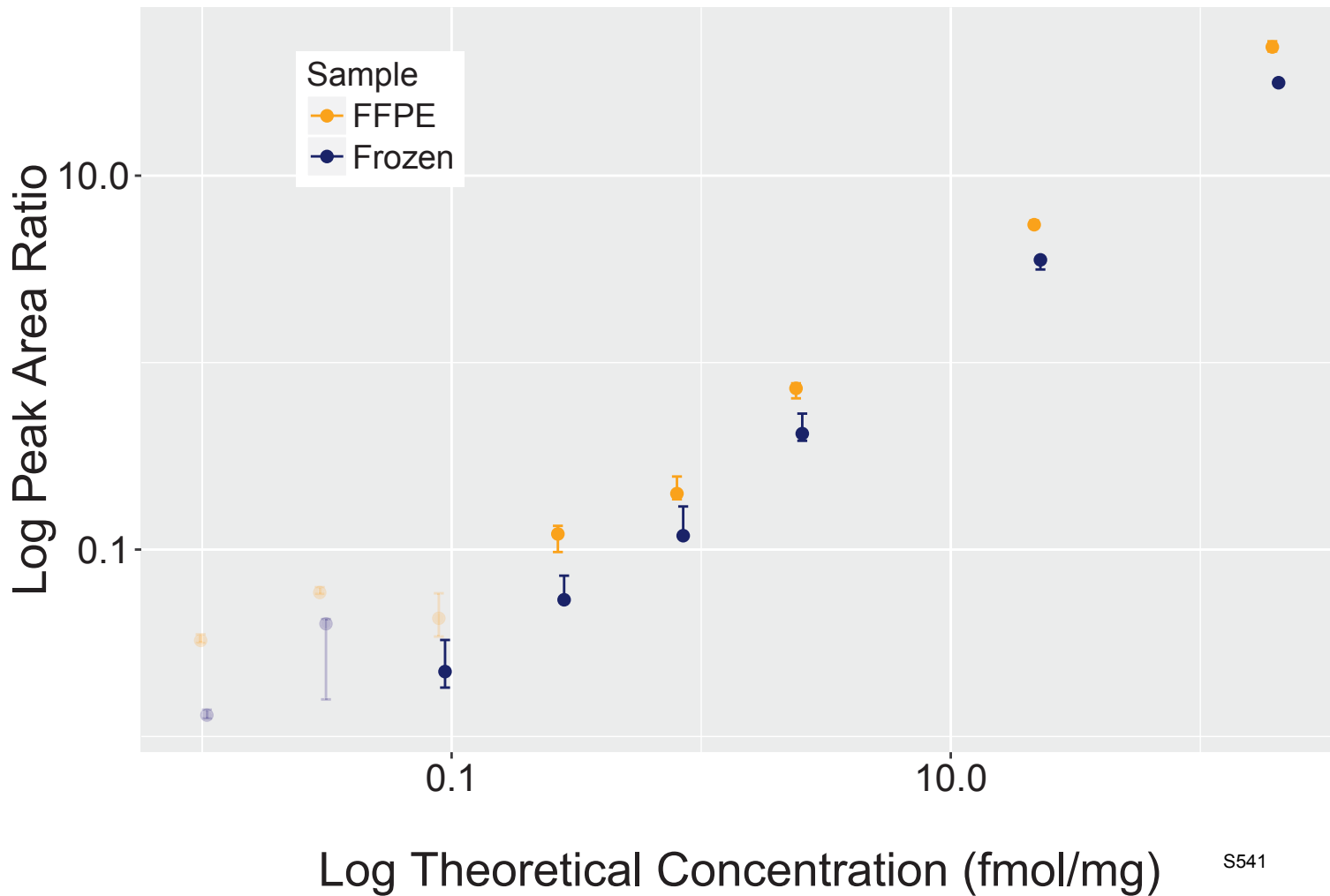
Analyte: CAPNS1.THYSNIEANEESEVR



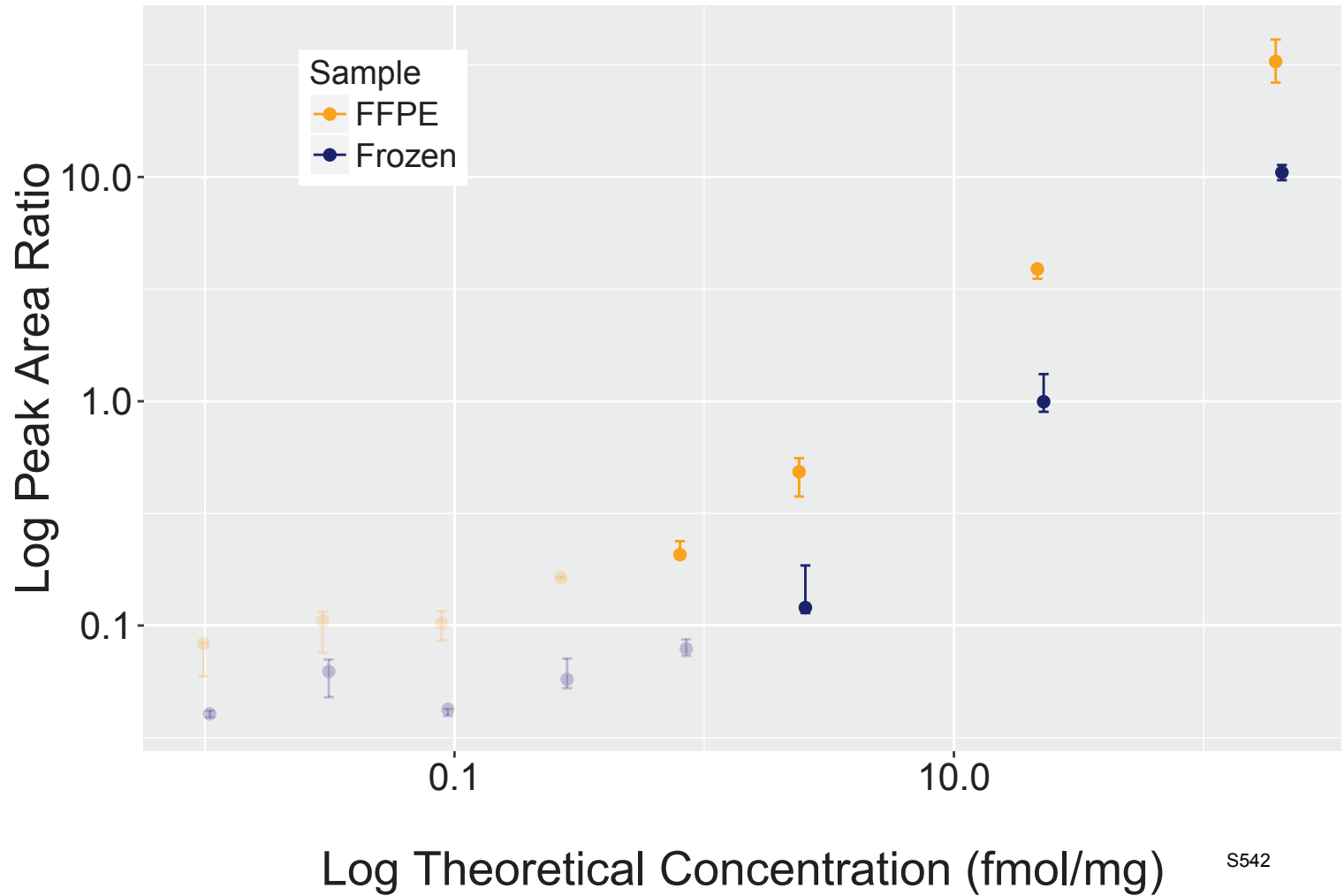
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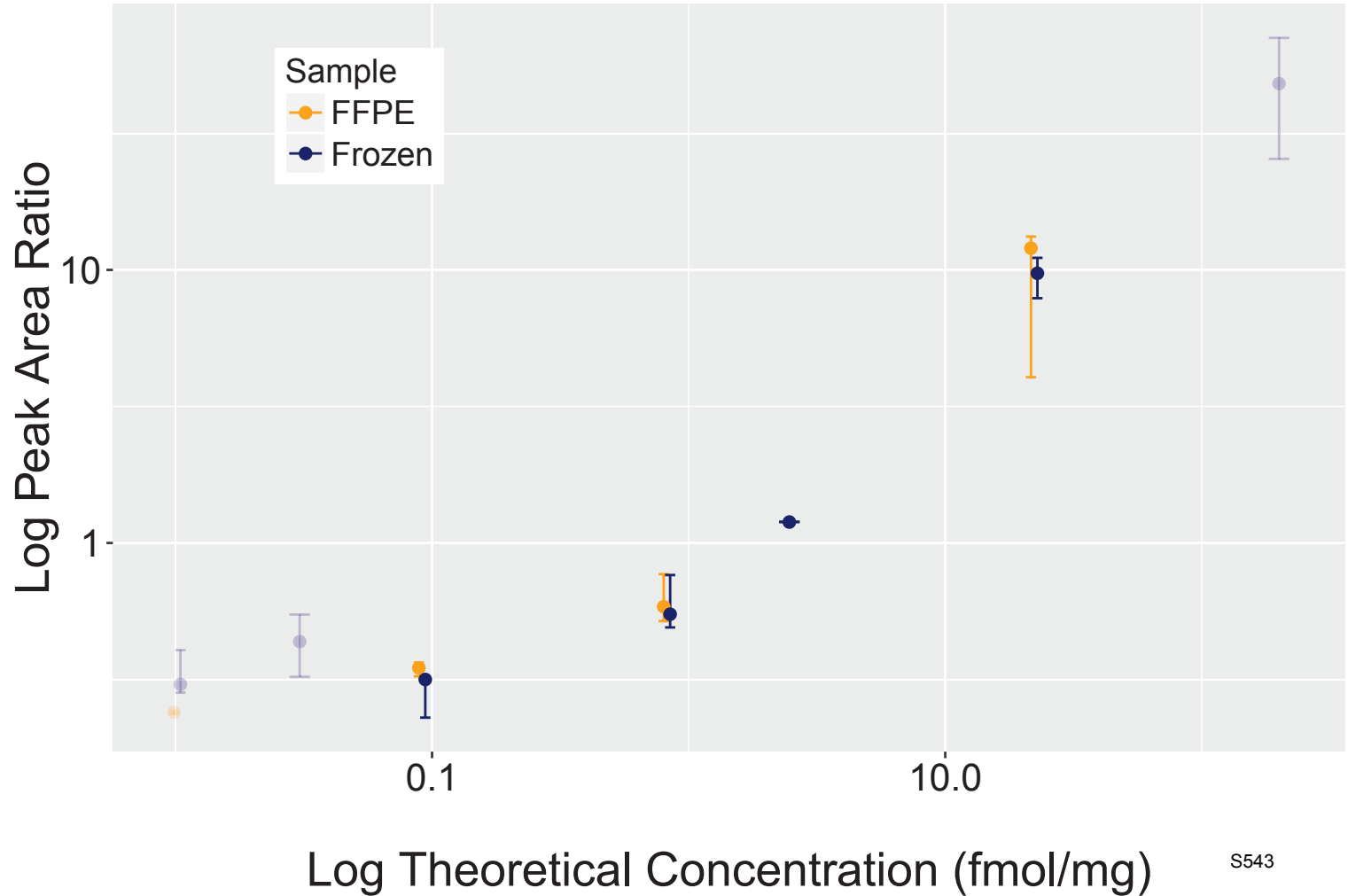
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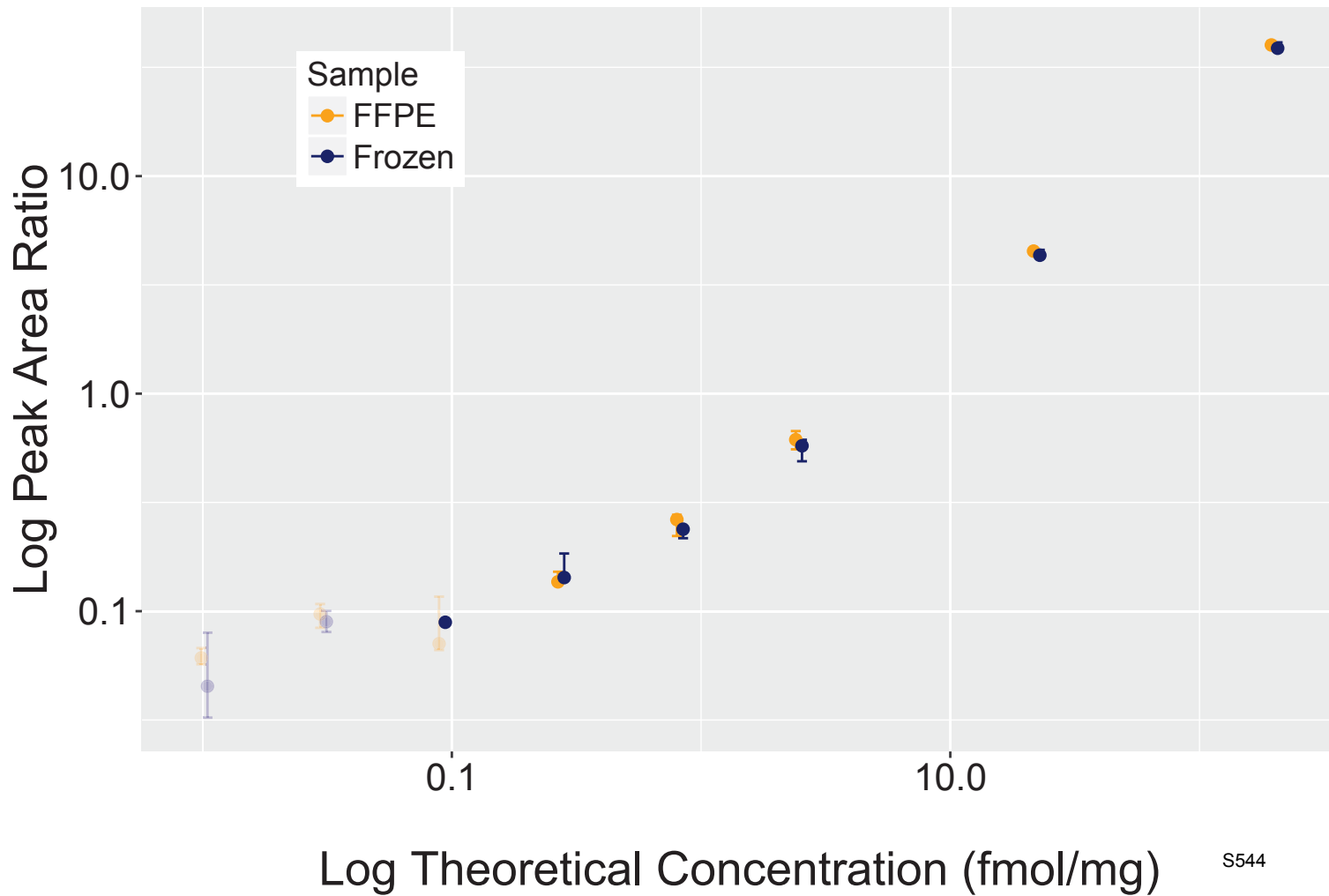
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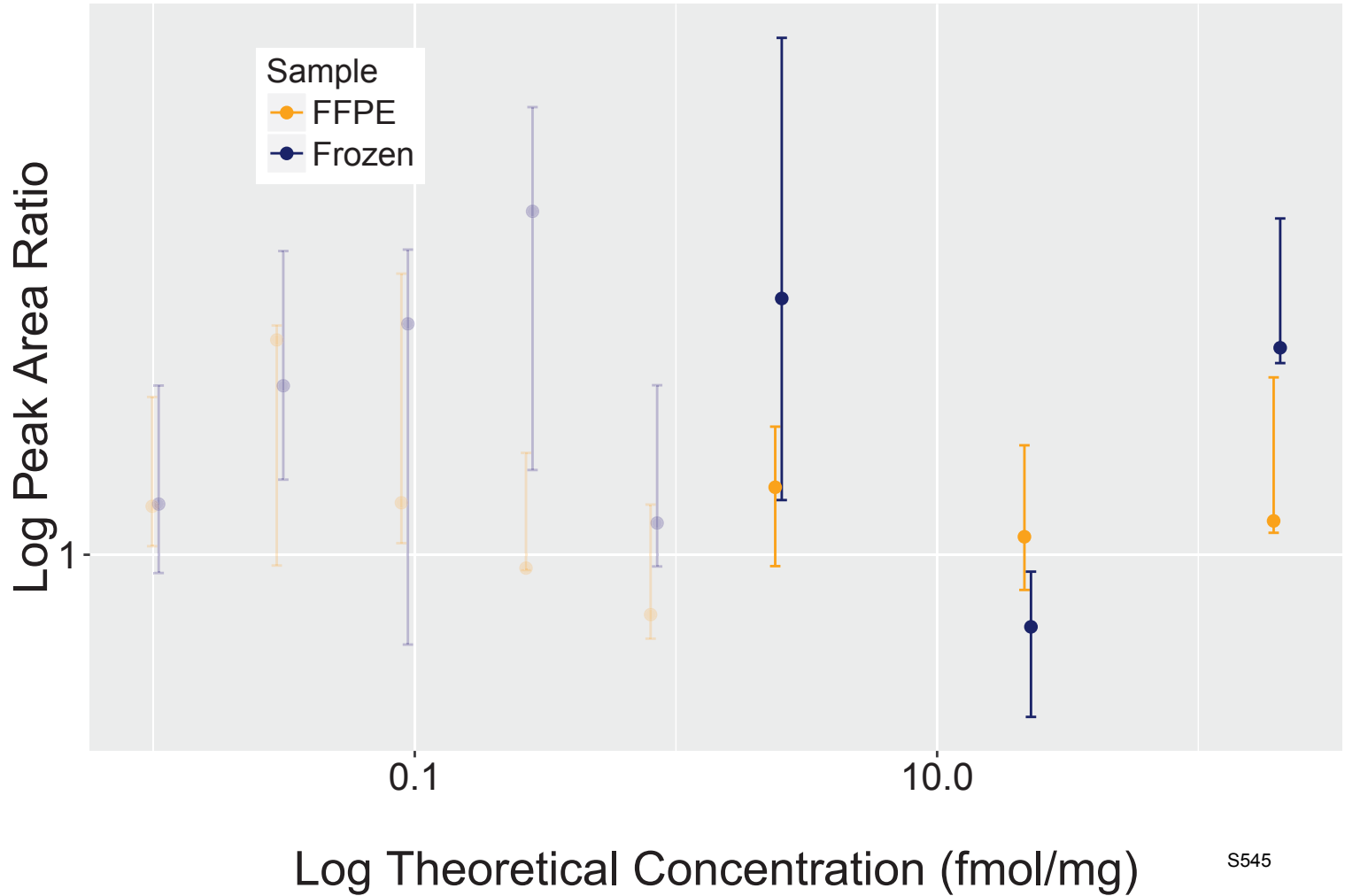
Analyte: HP1BP3.TIPSWATLSASQLAR



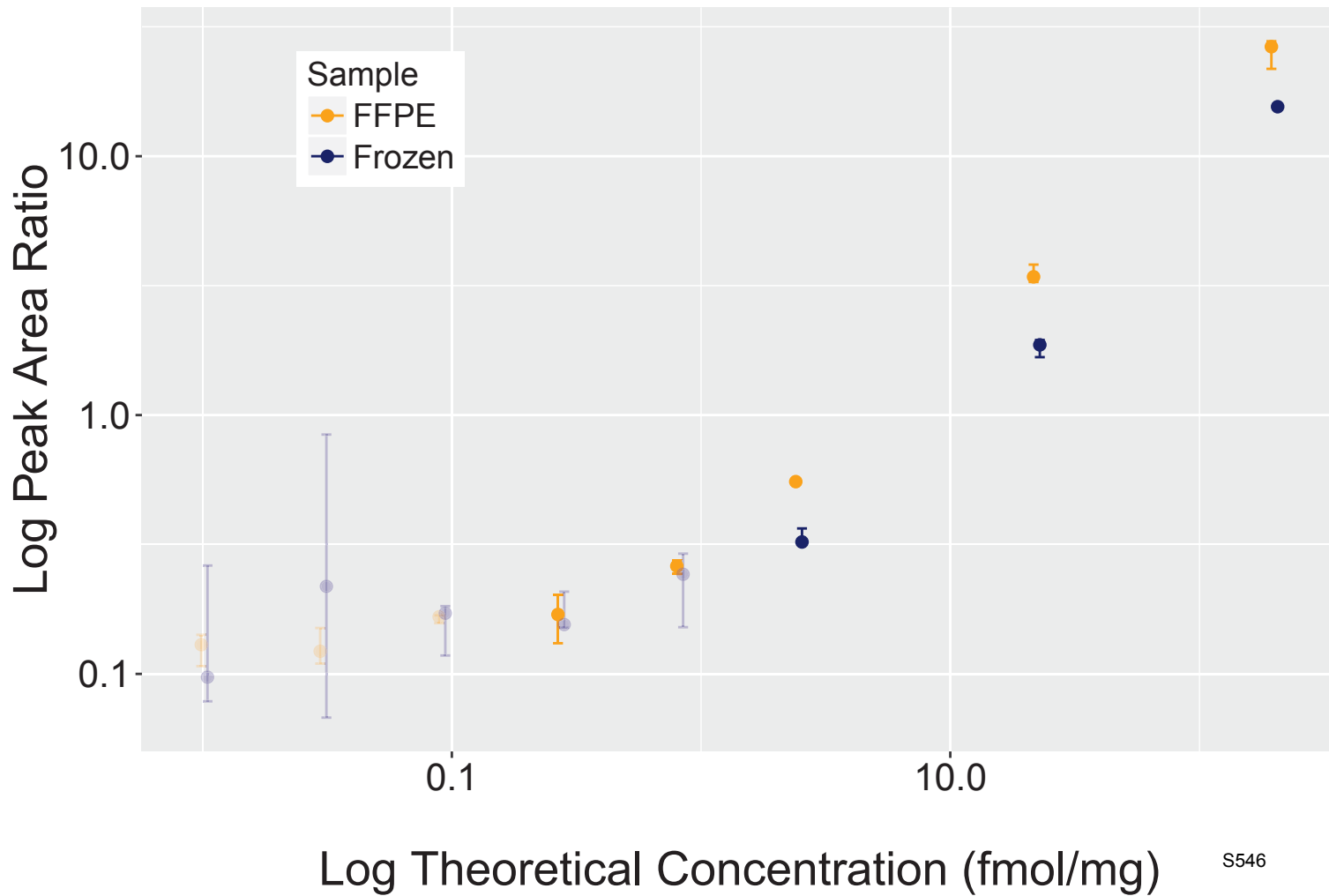
Analyte: AARS.TITVALADGGRPDNTGR



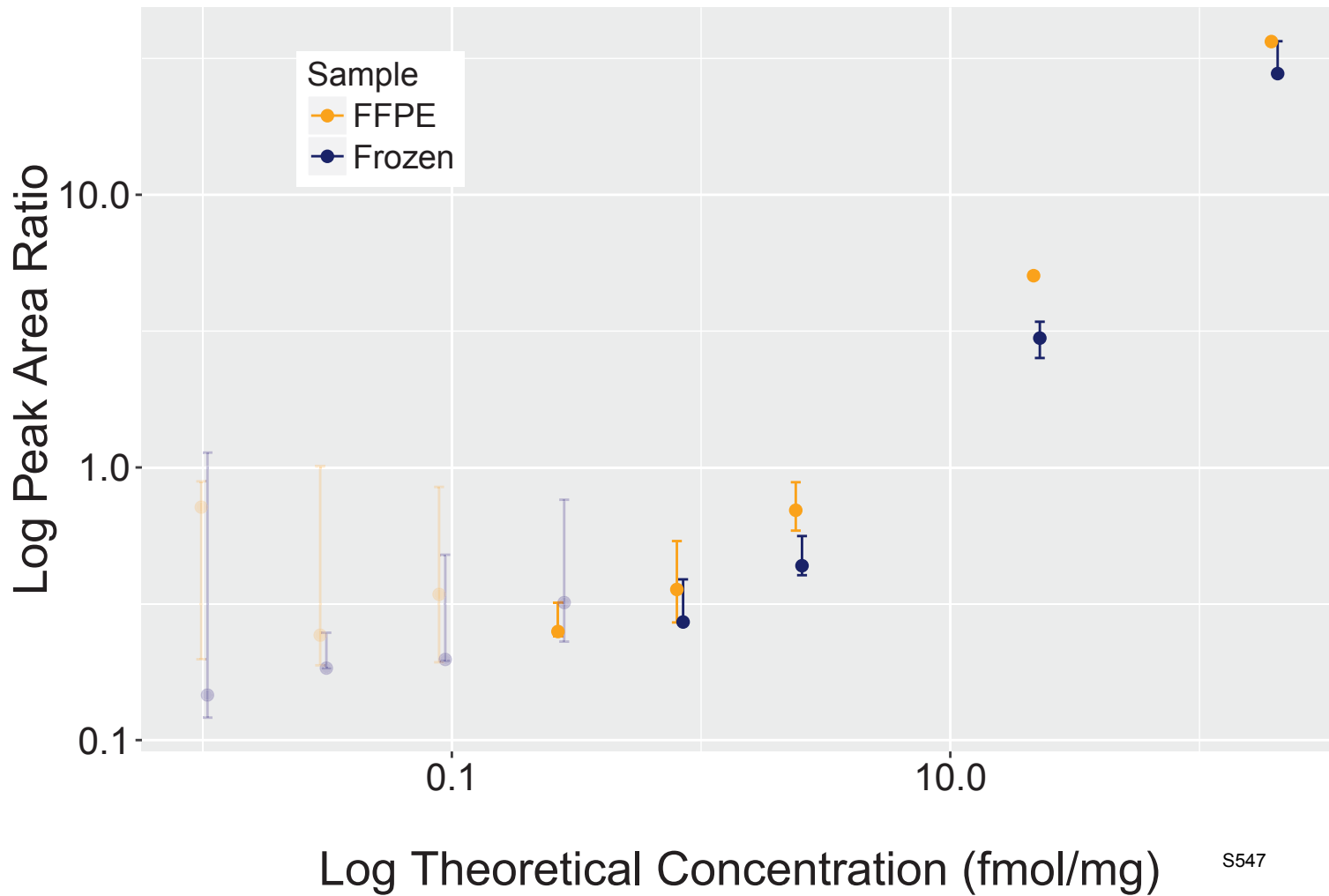
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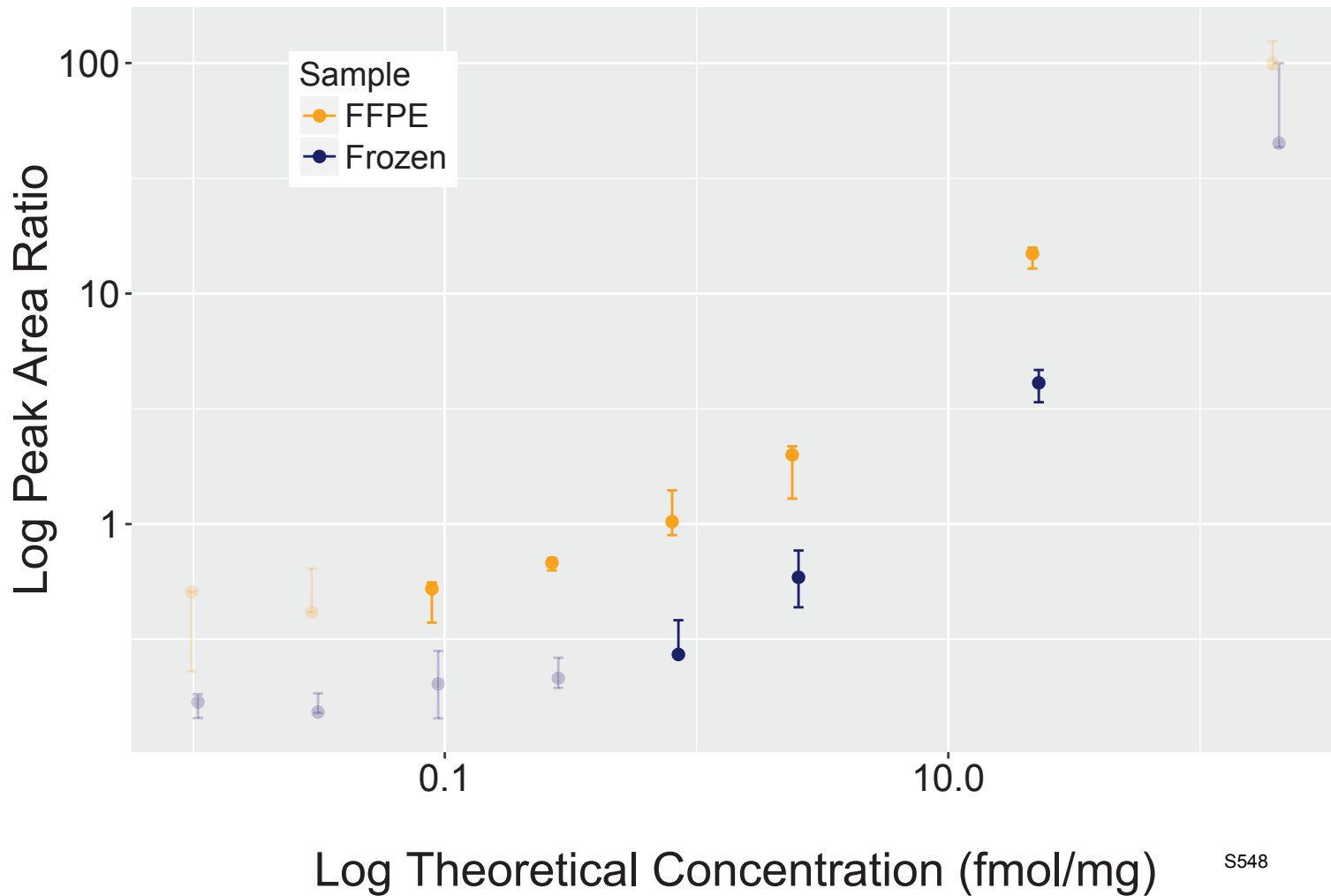
Analyte: HADHA.TLQEVTLQLSQEAQR



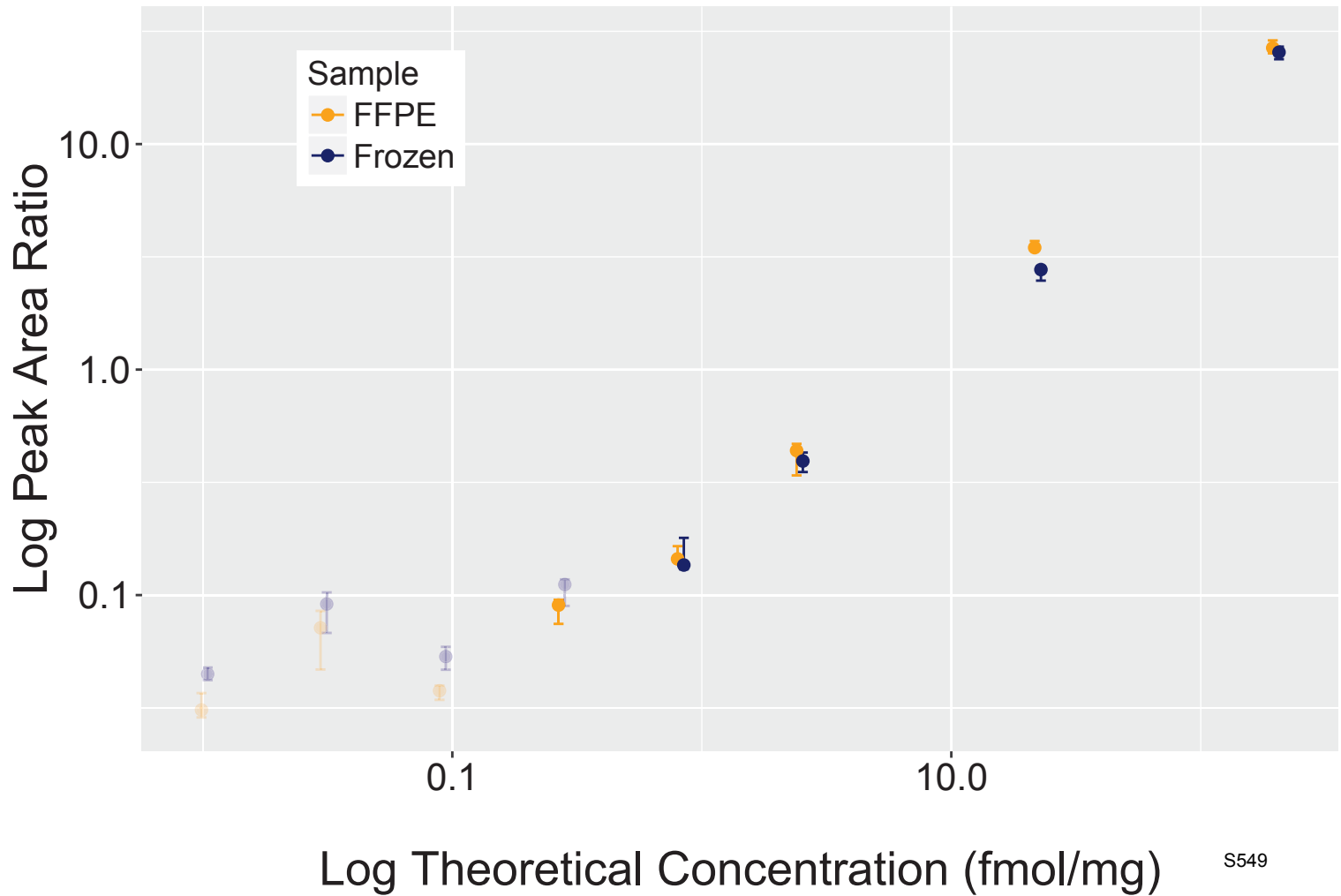
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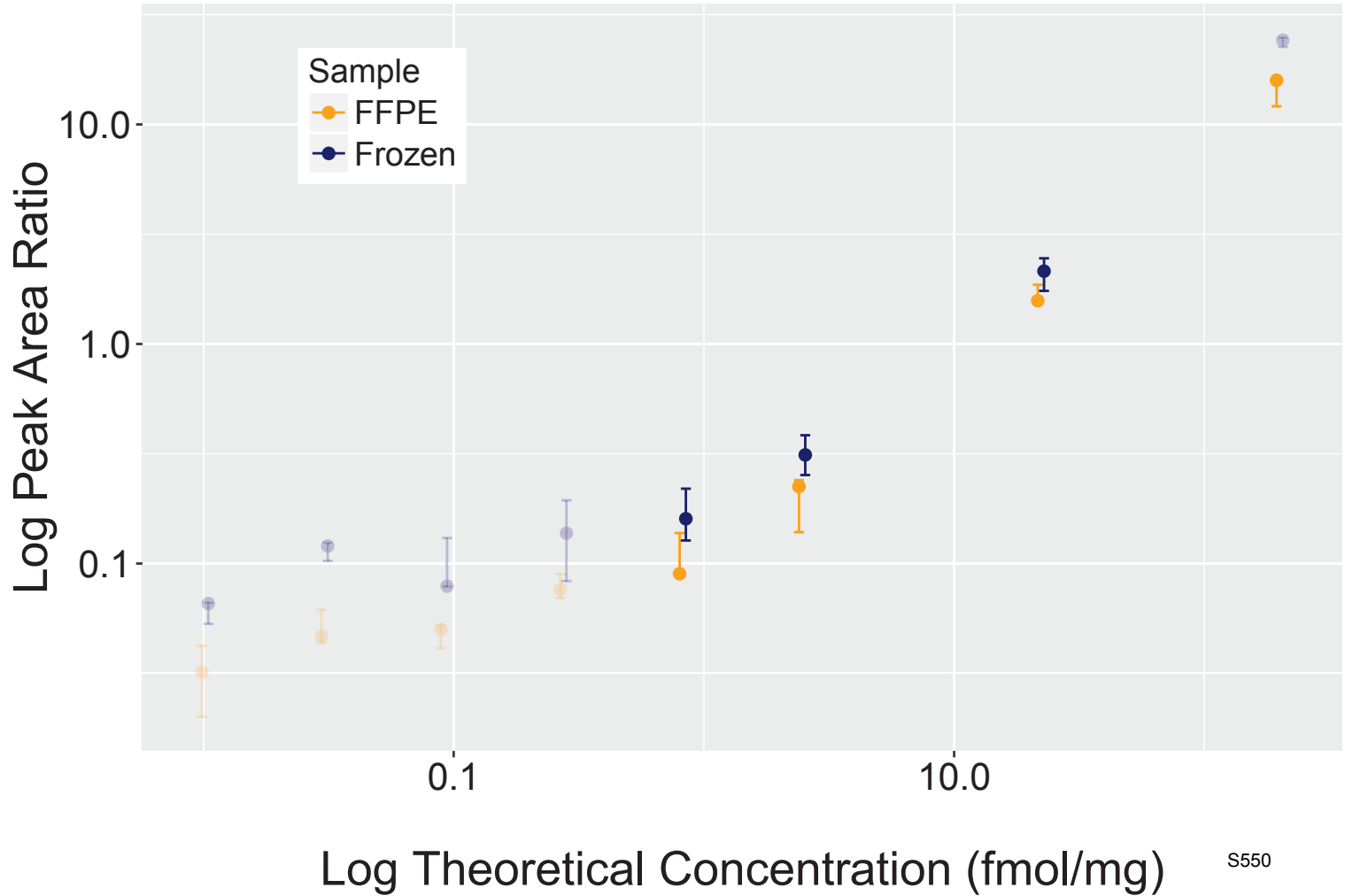
Analyte: NIT2.TLSPGDSFSTFDTPYC[+57]R



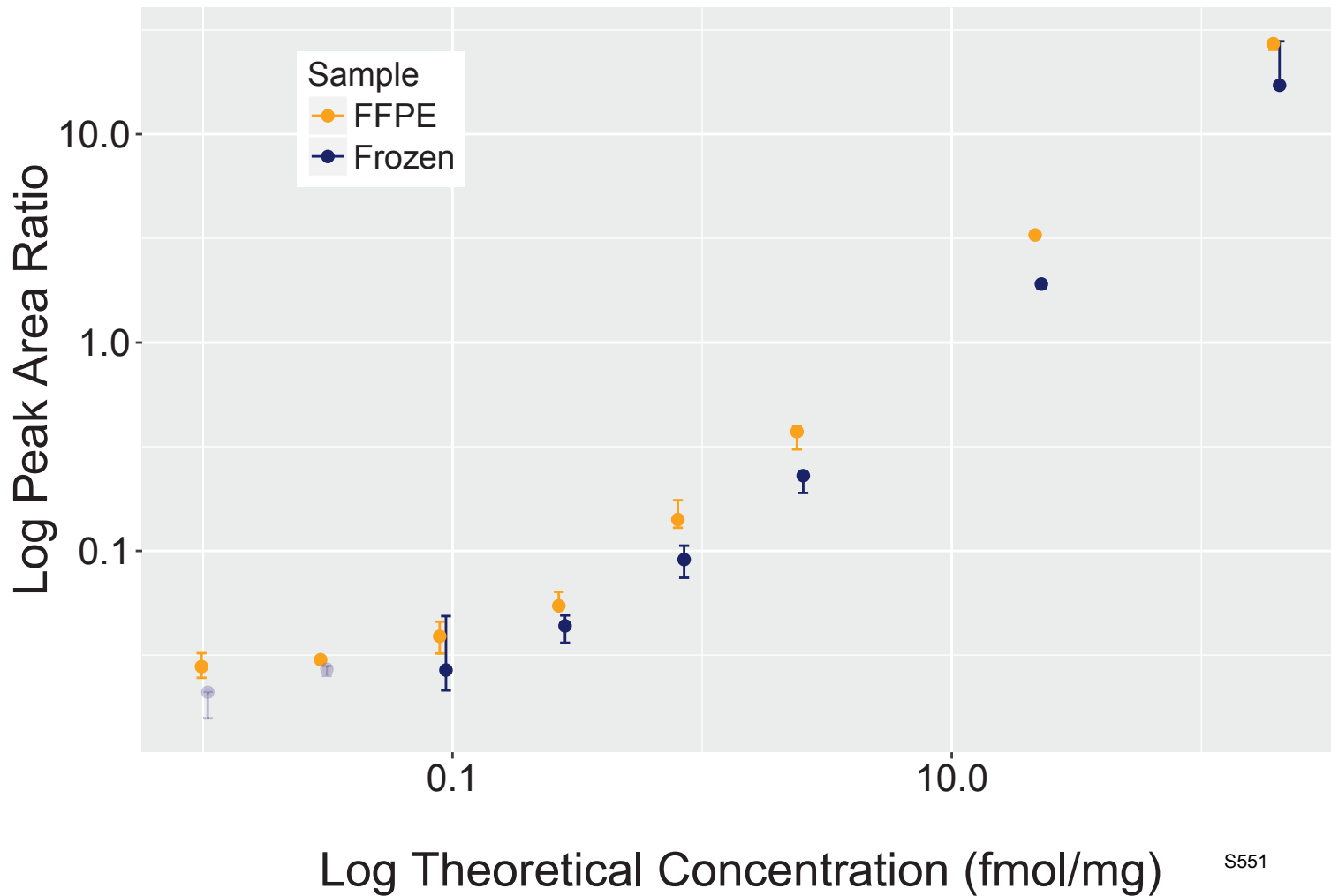
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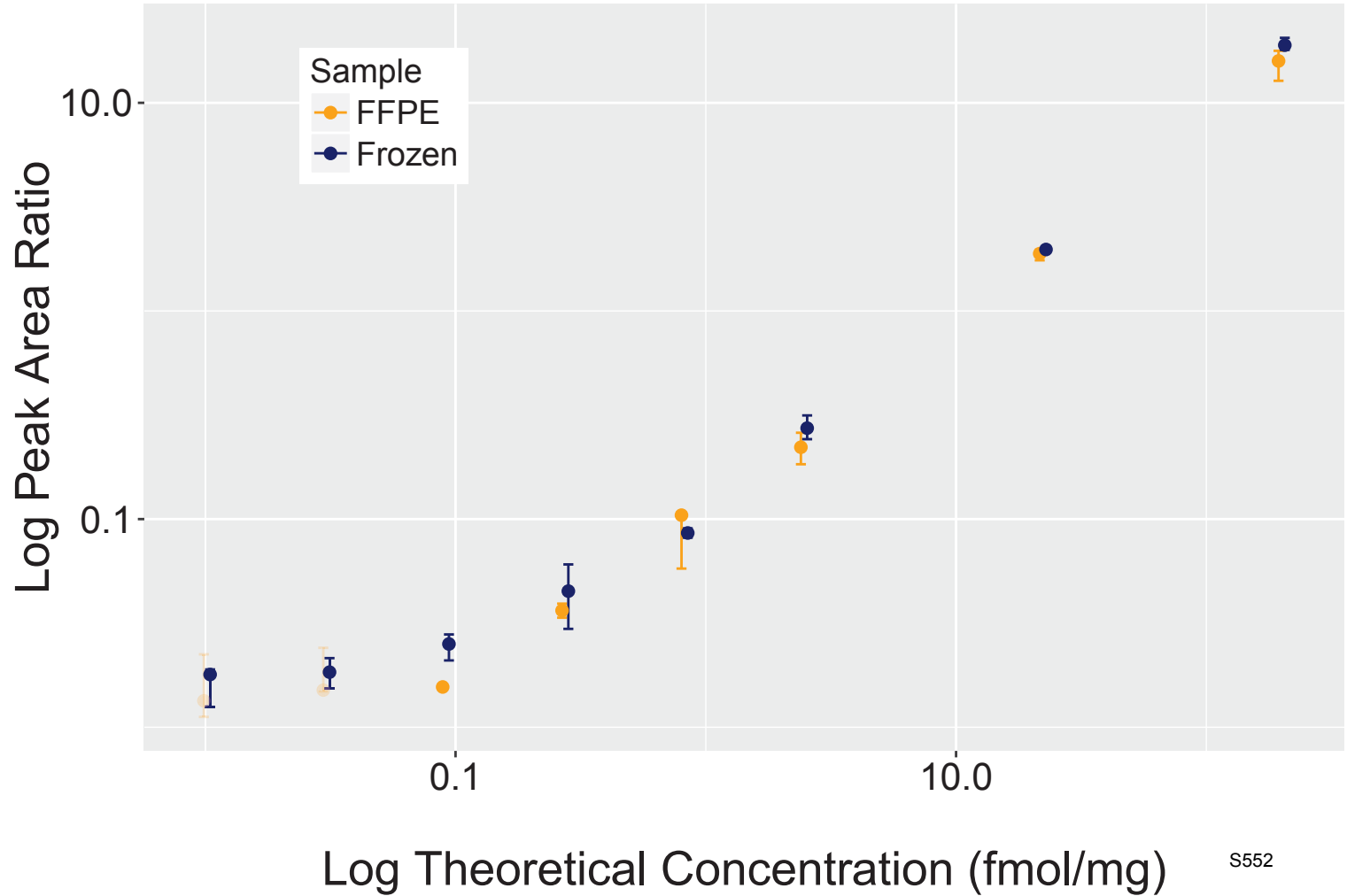
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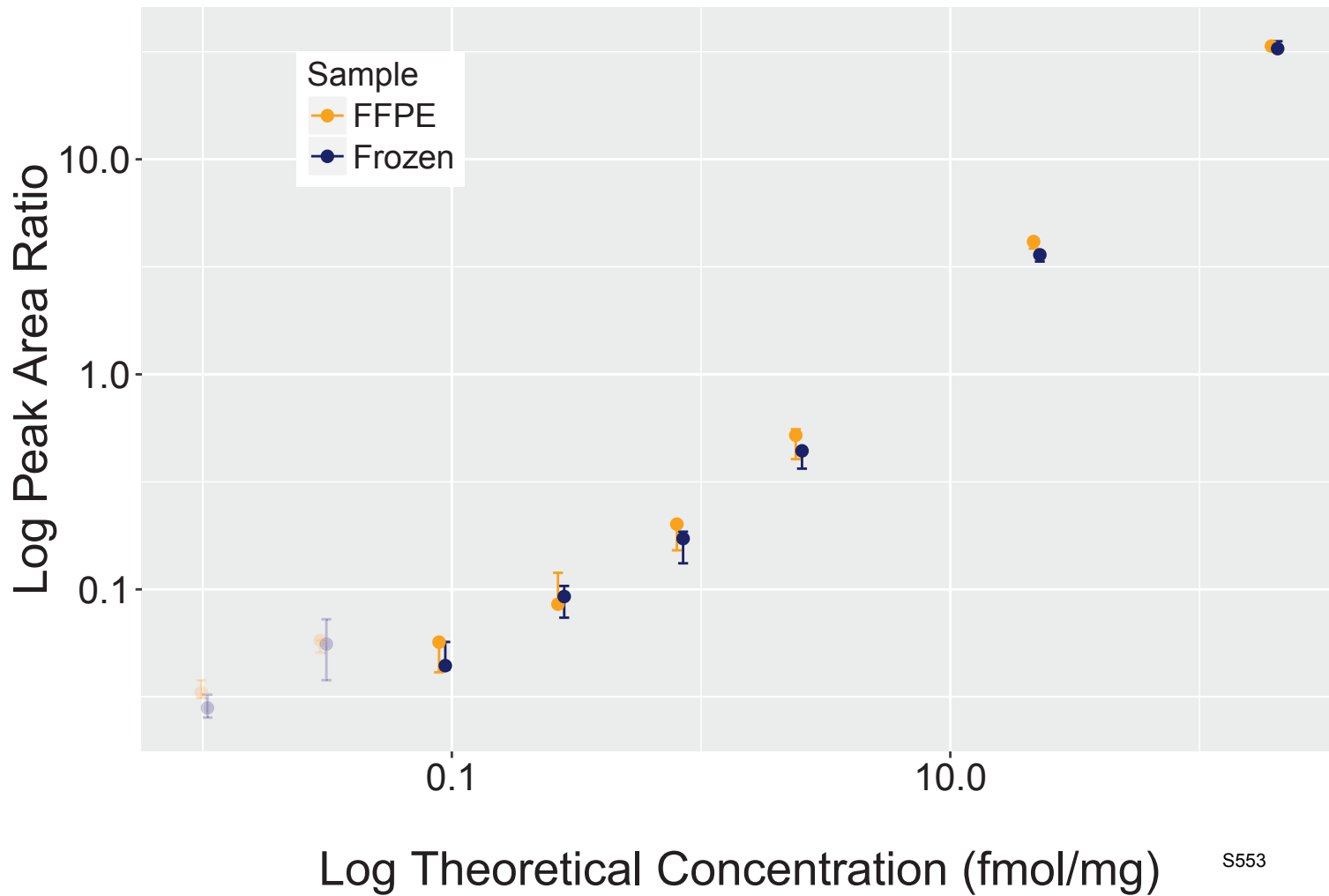
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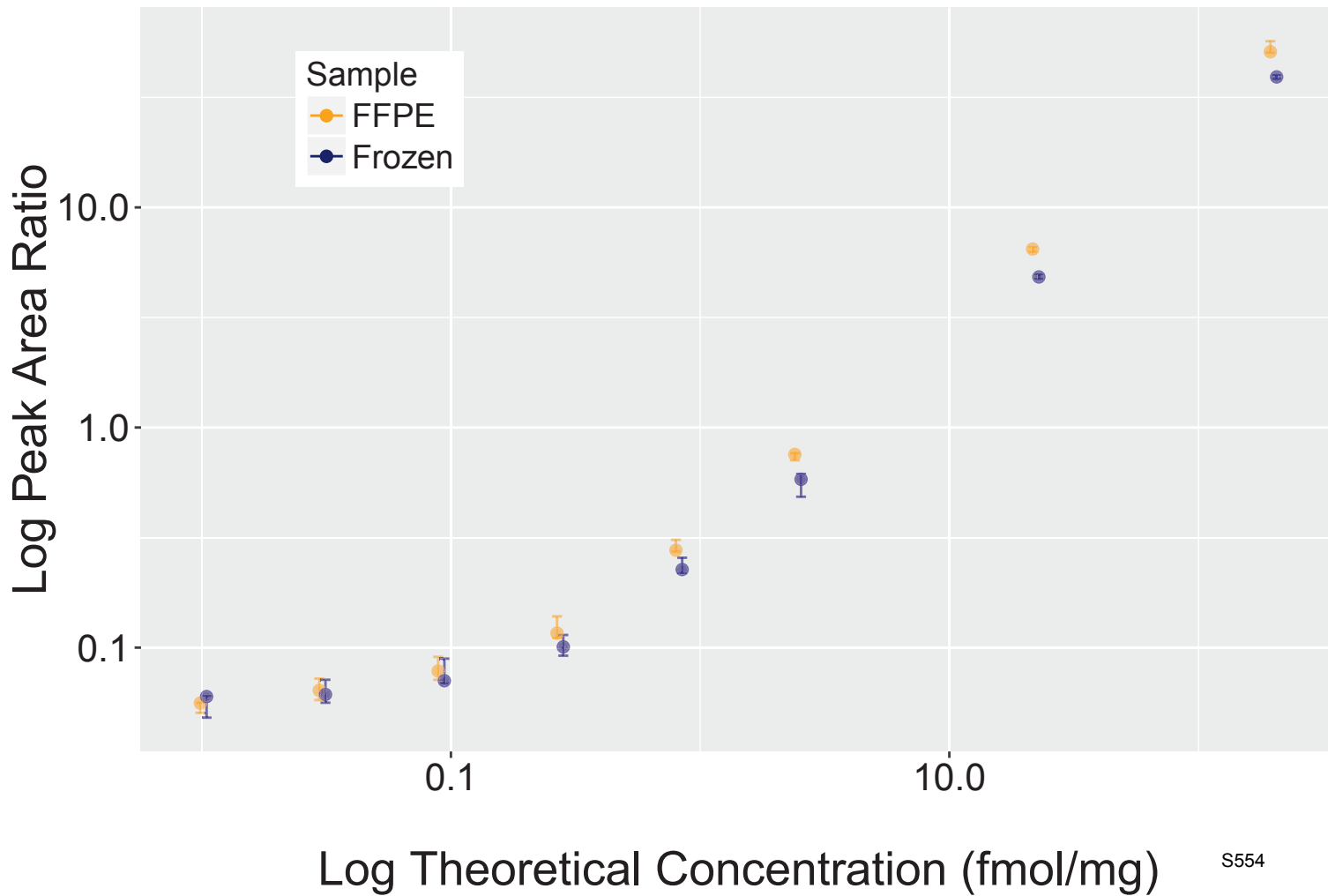
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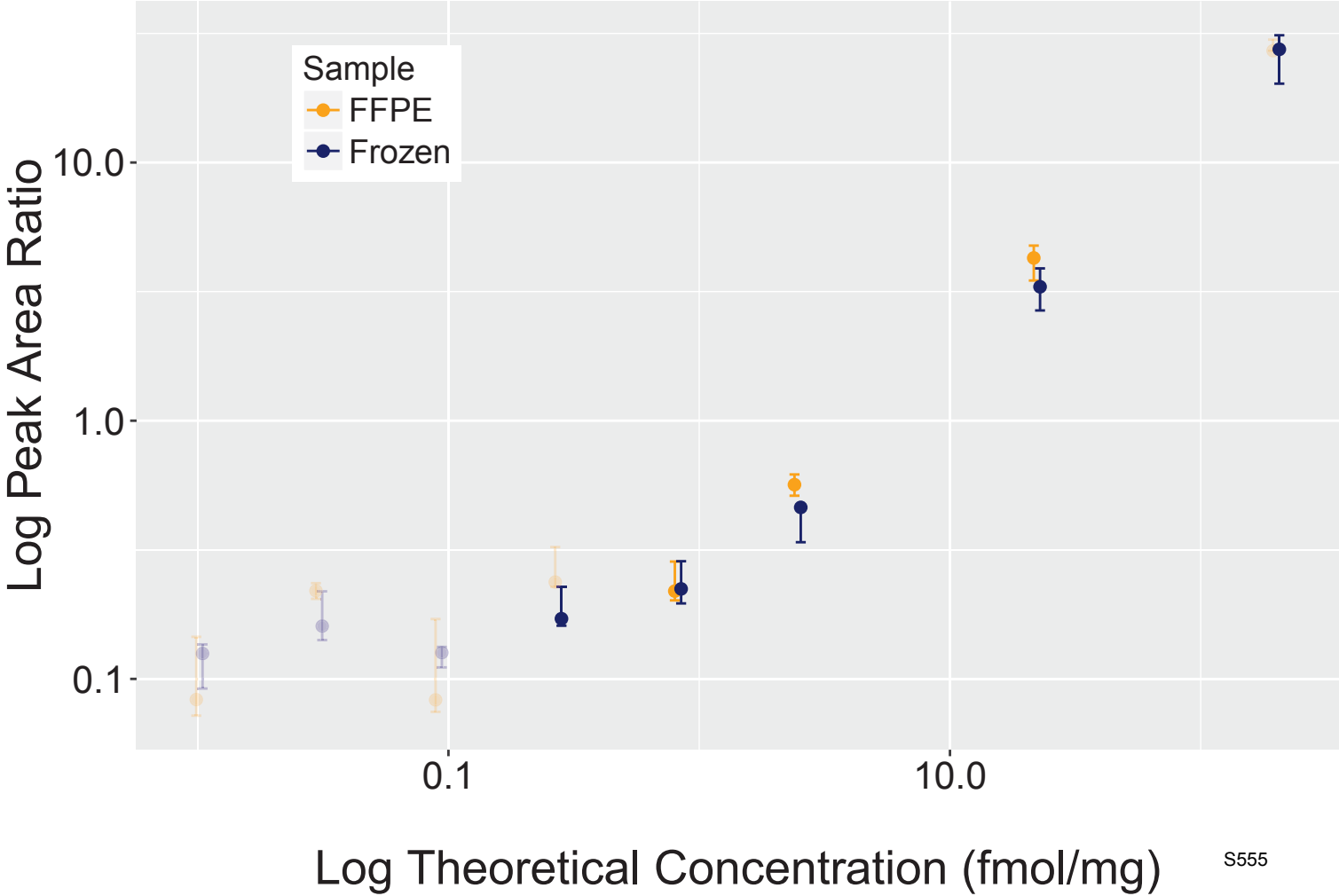
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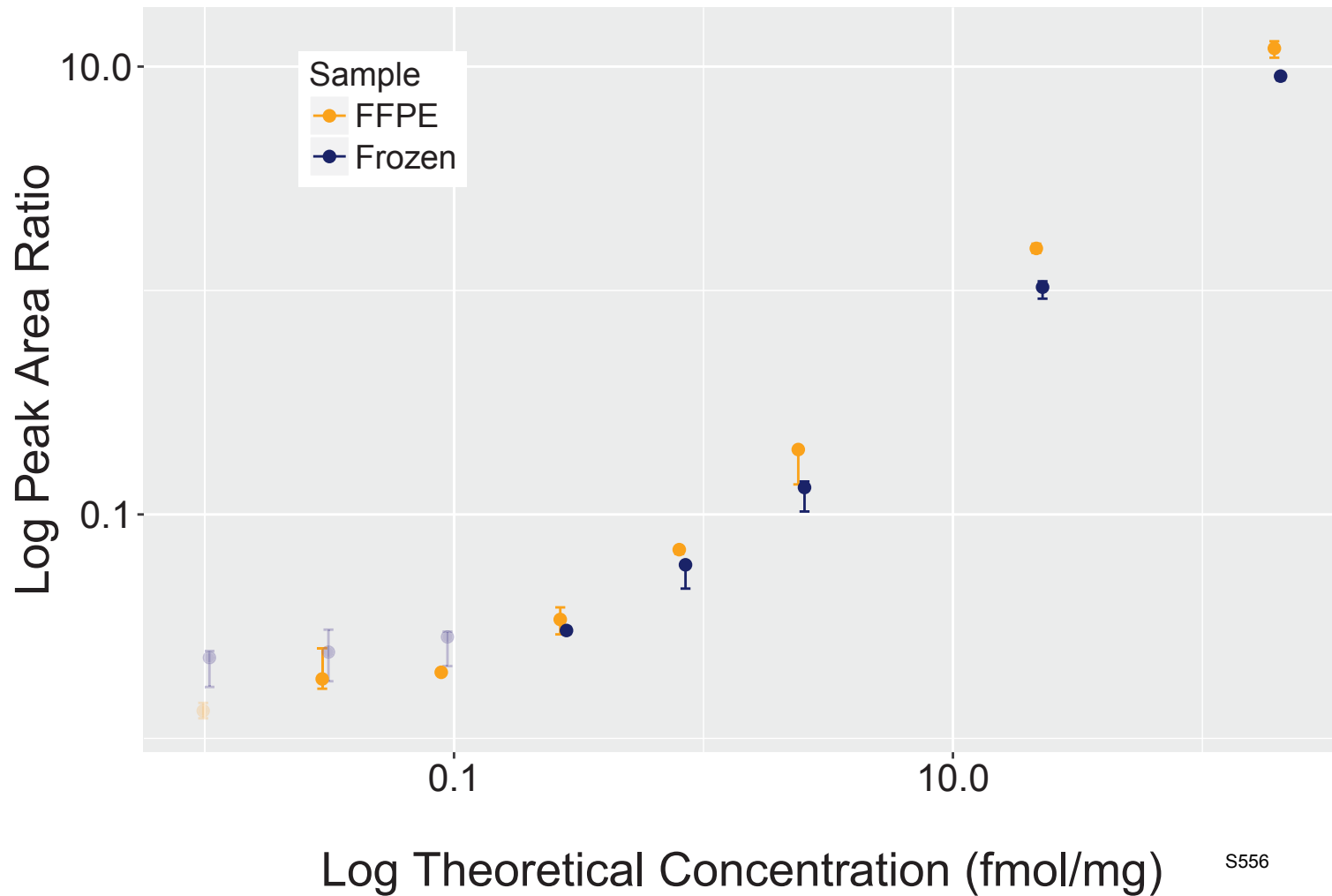
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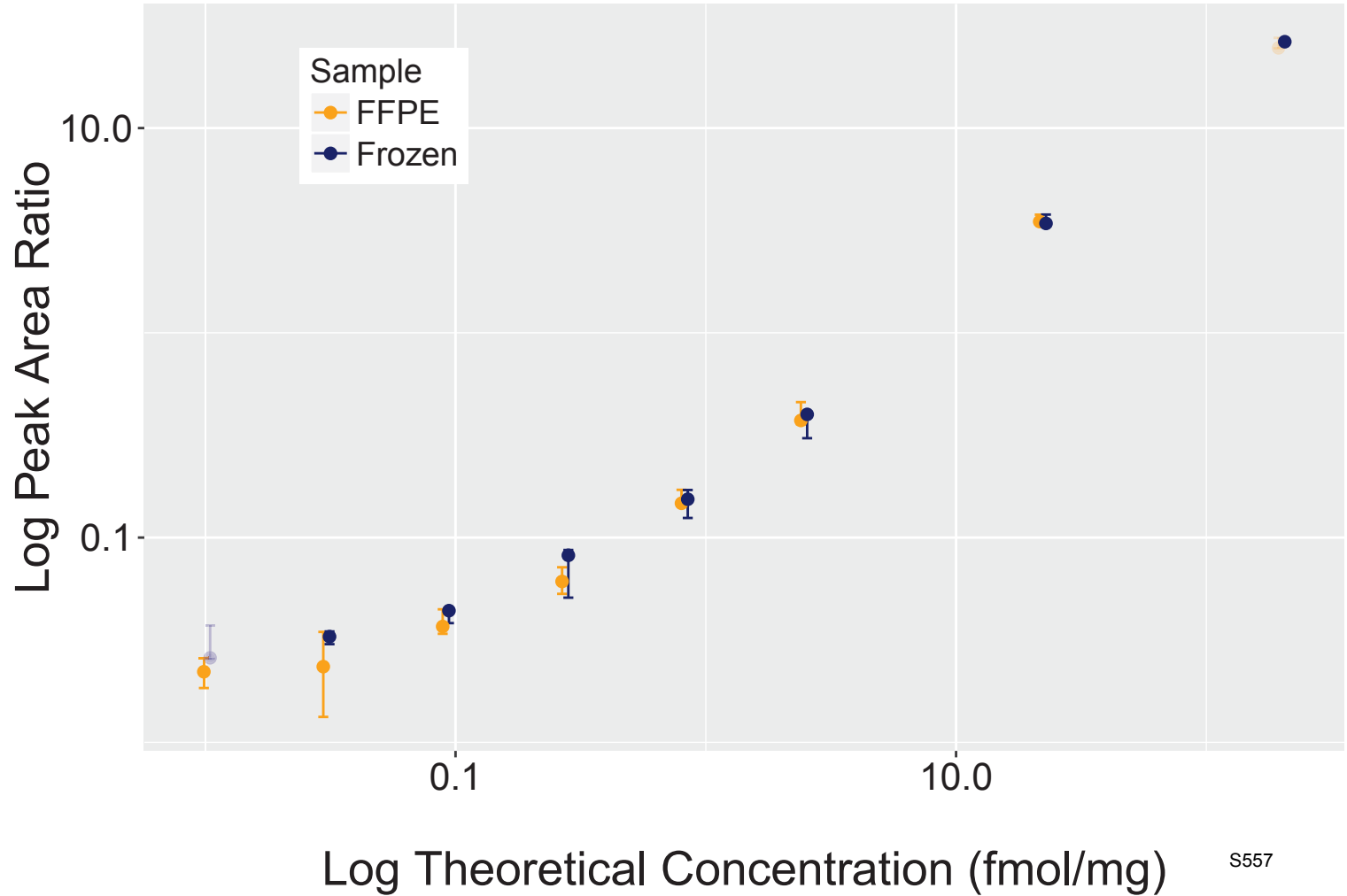
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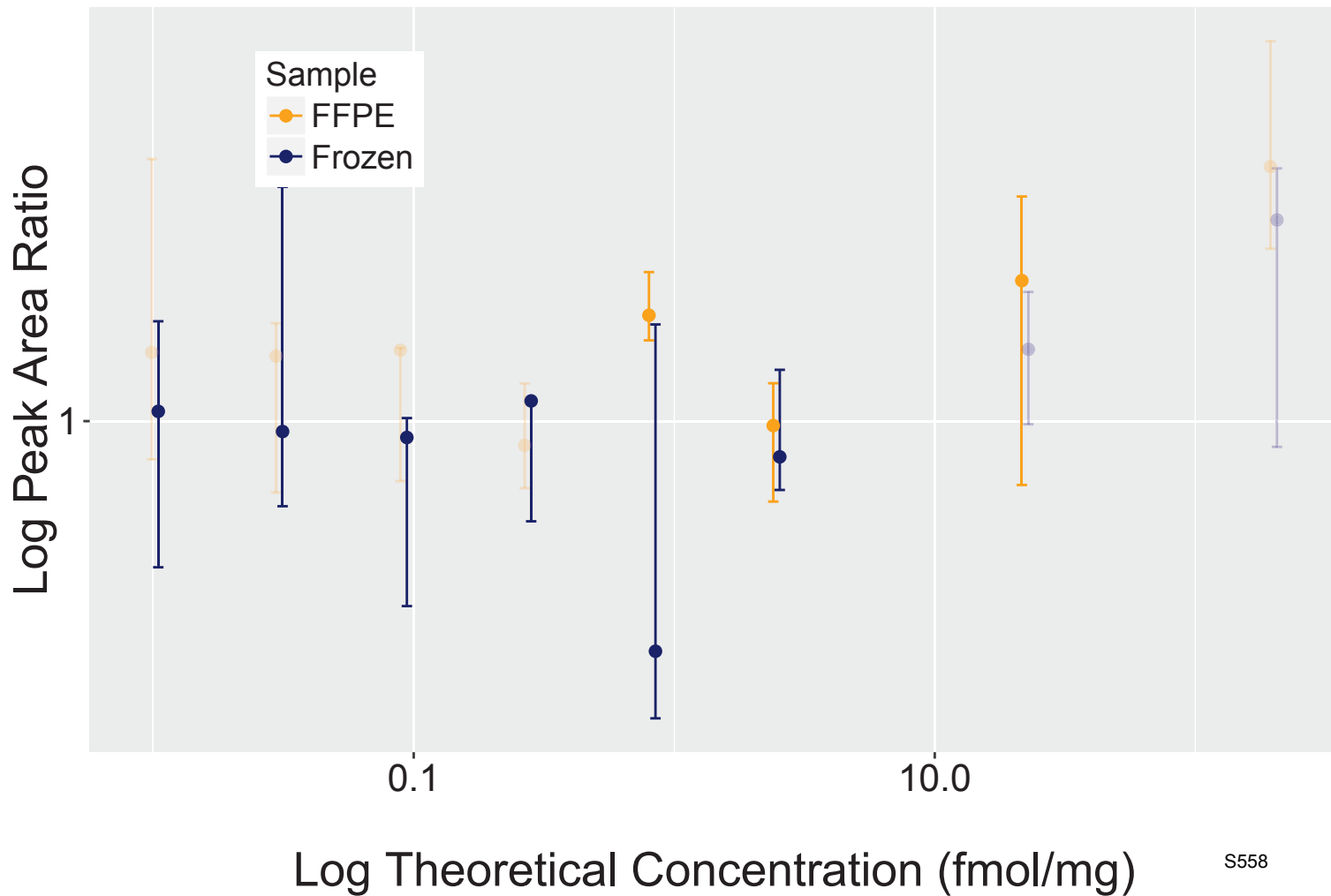
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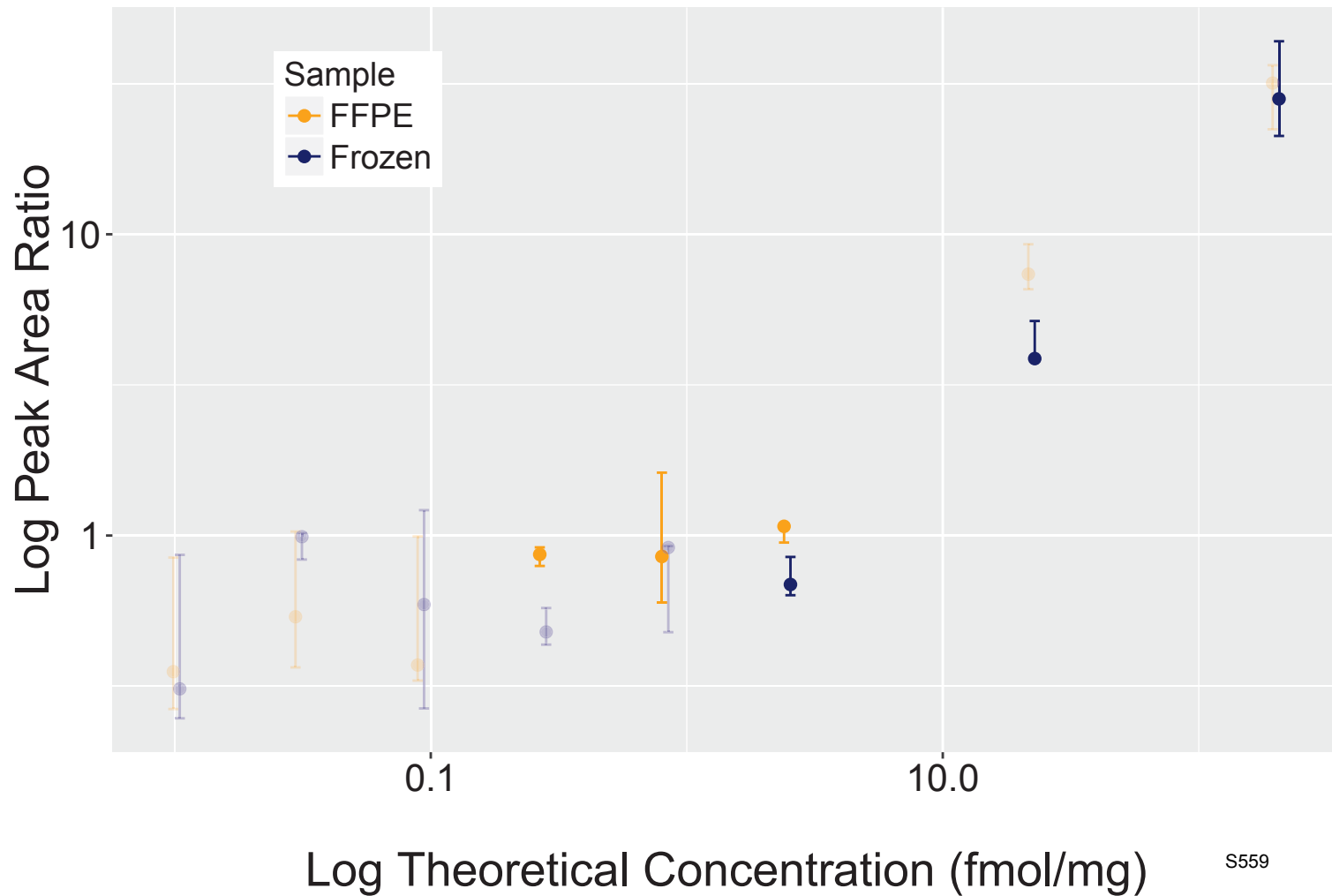
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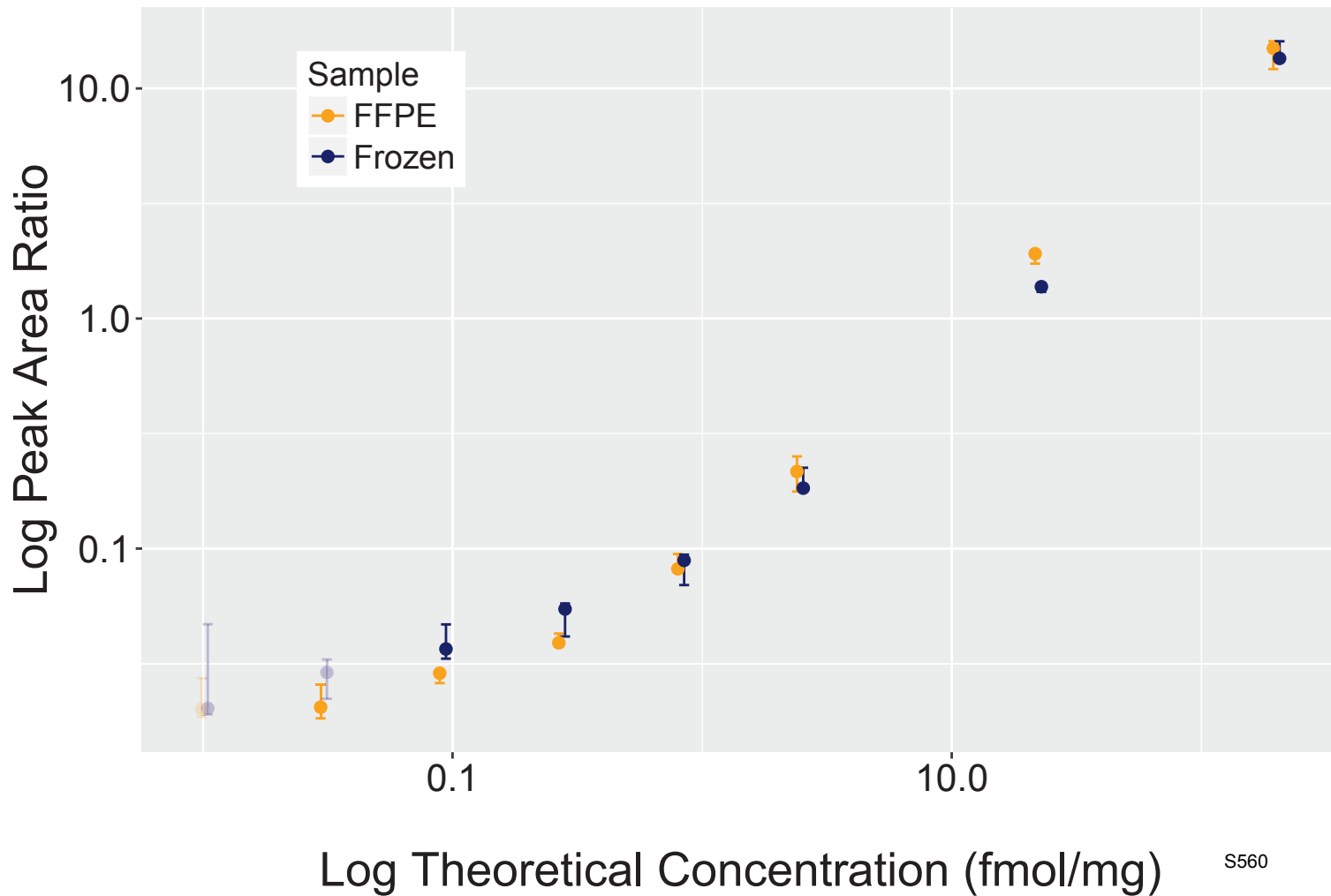
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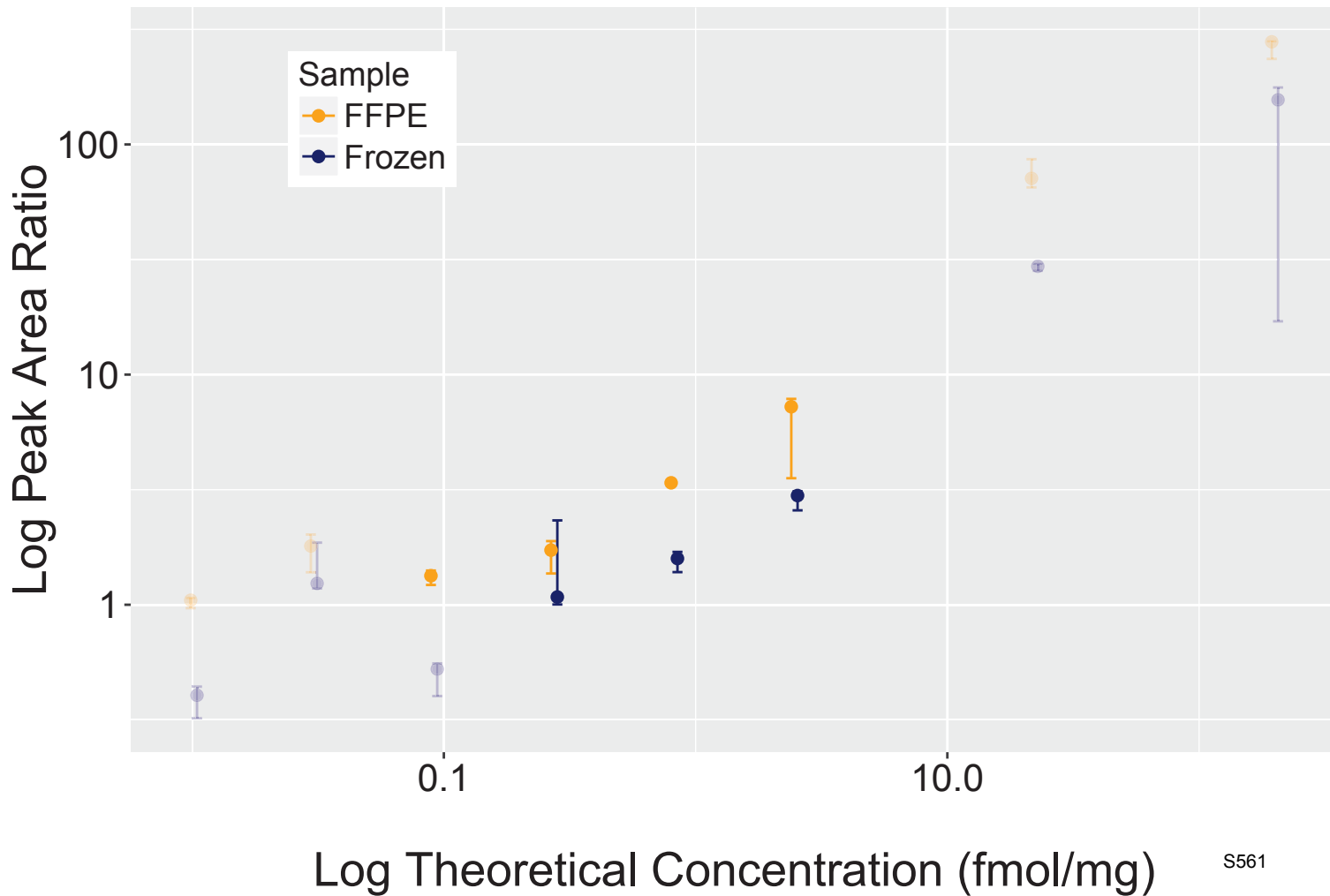
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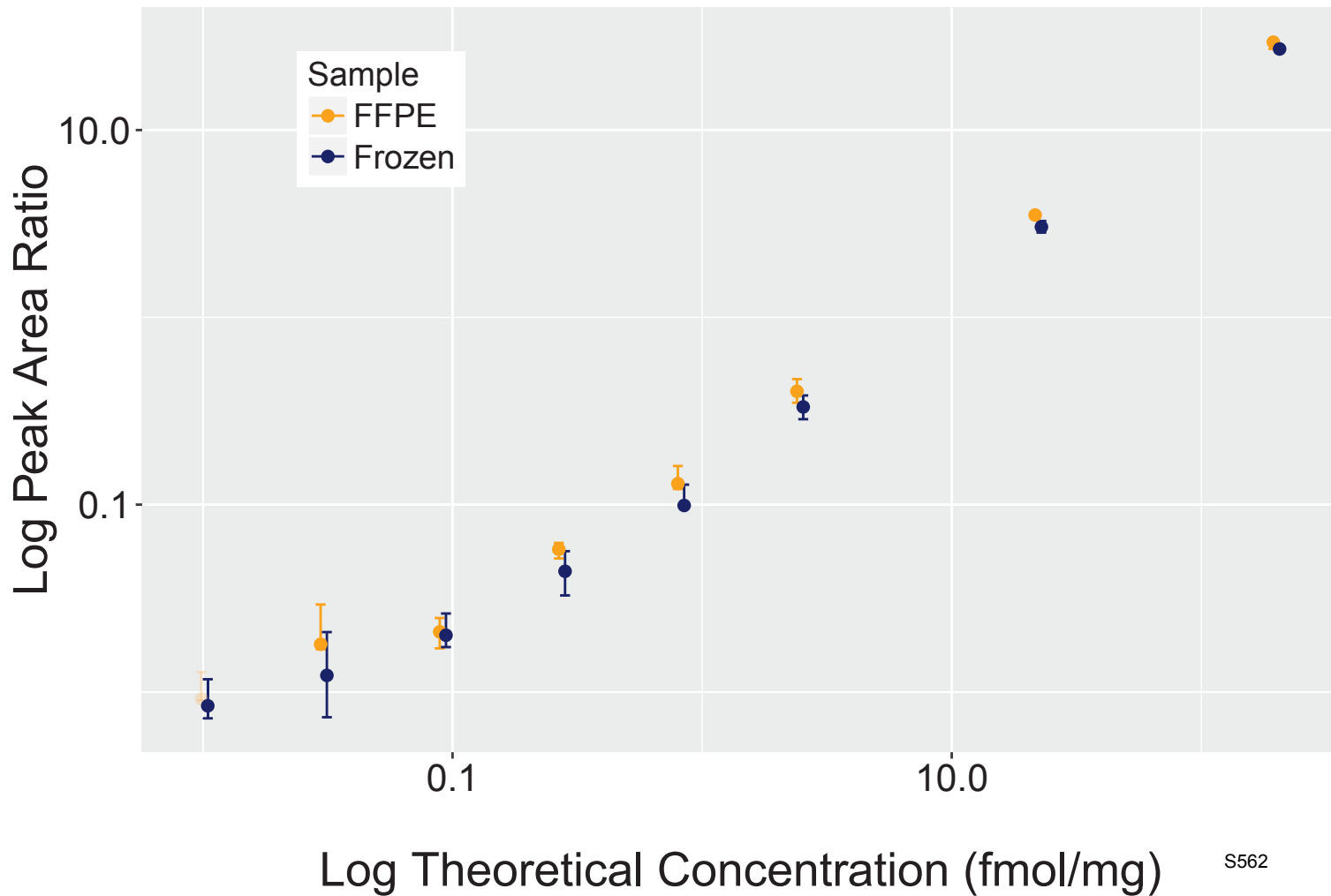
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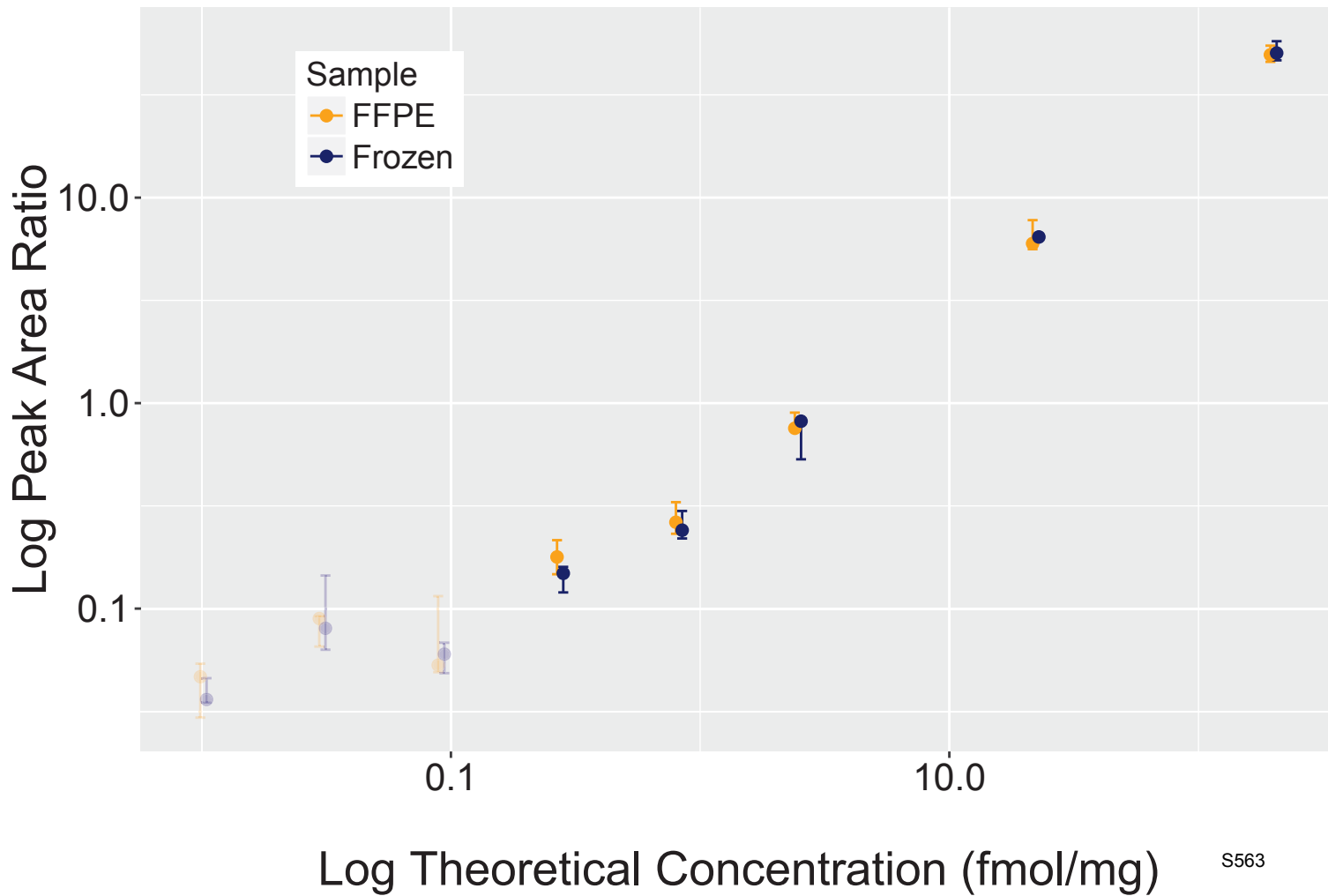
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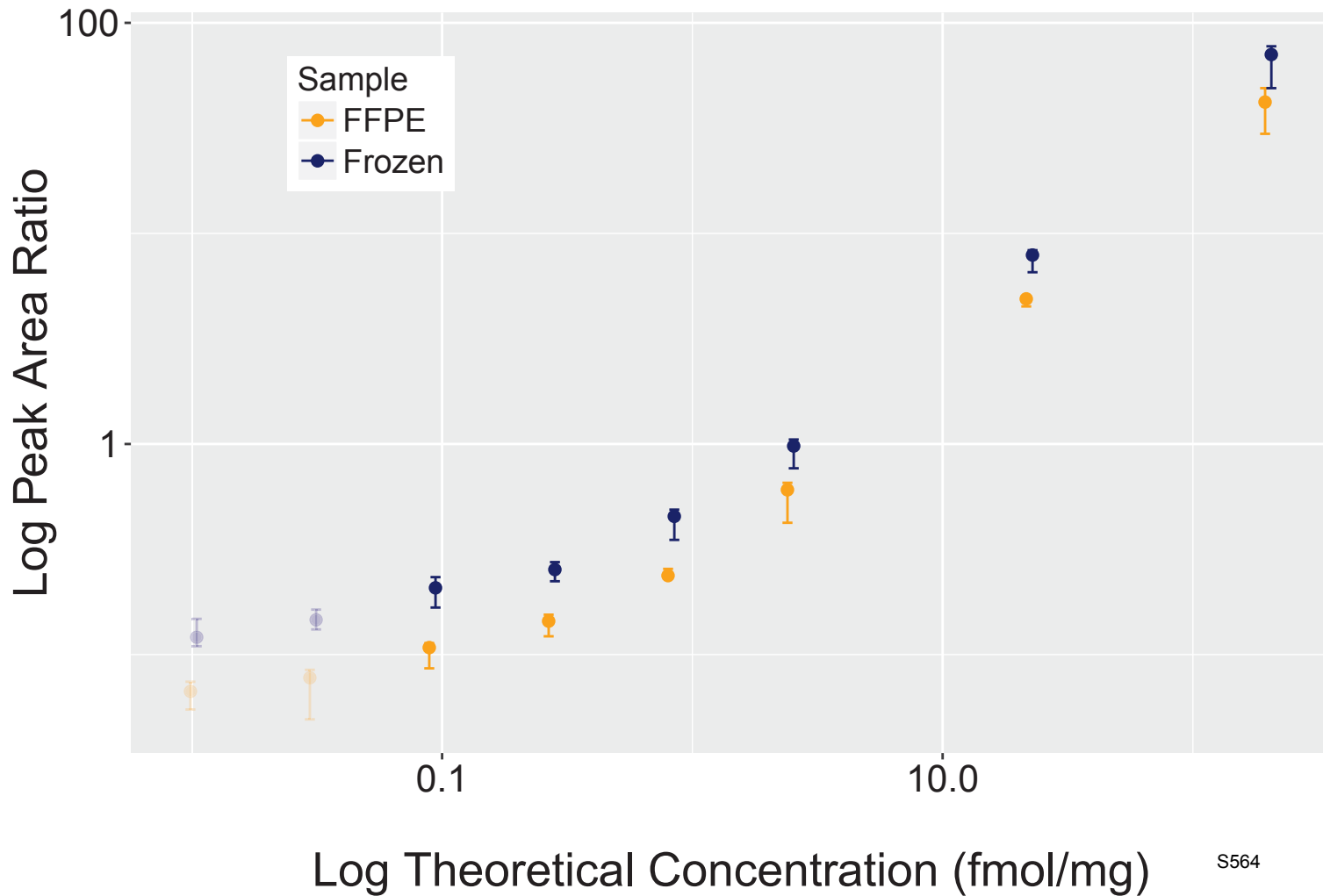
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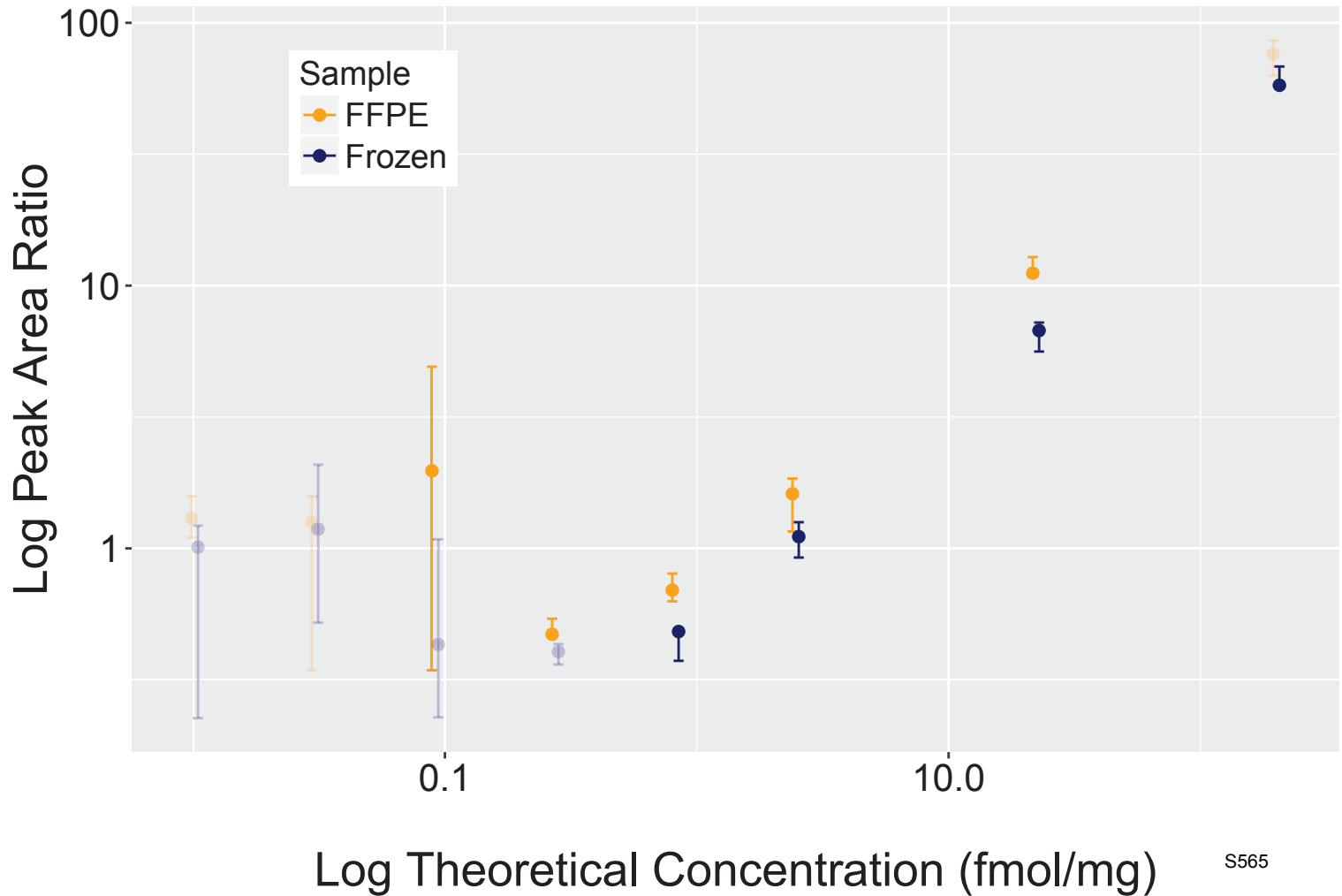
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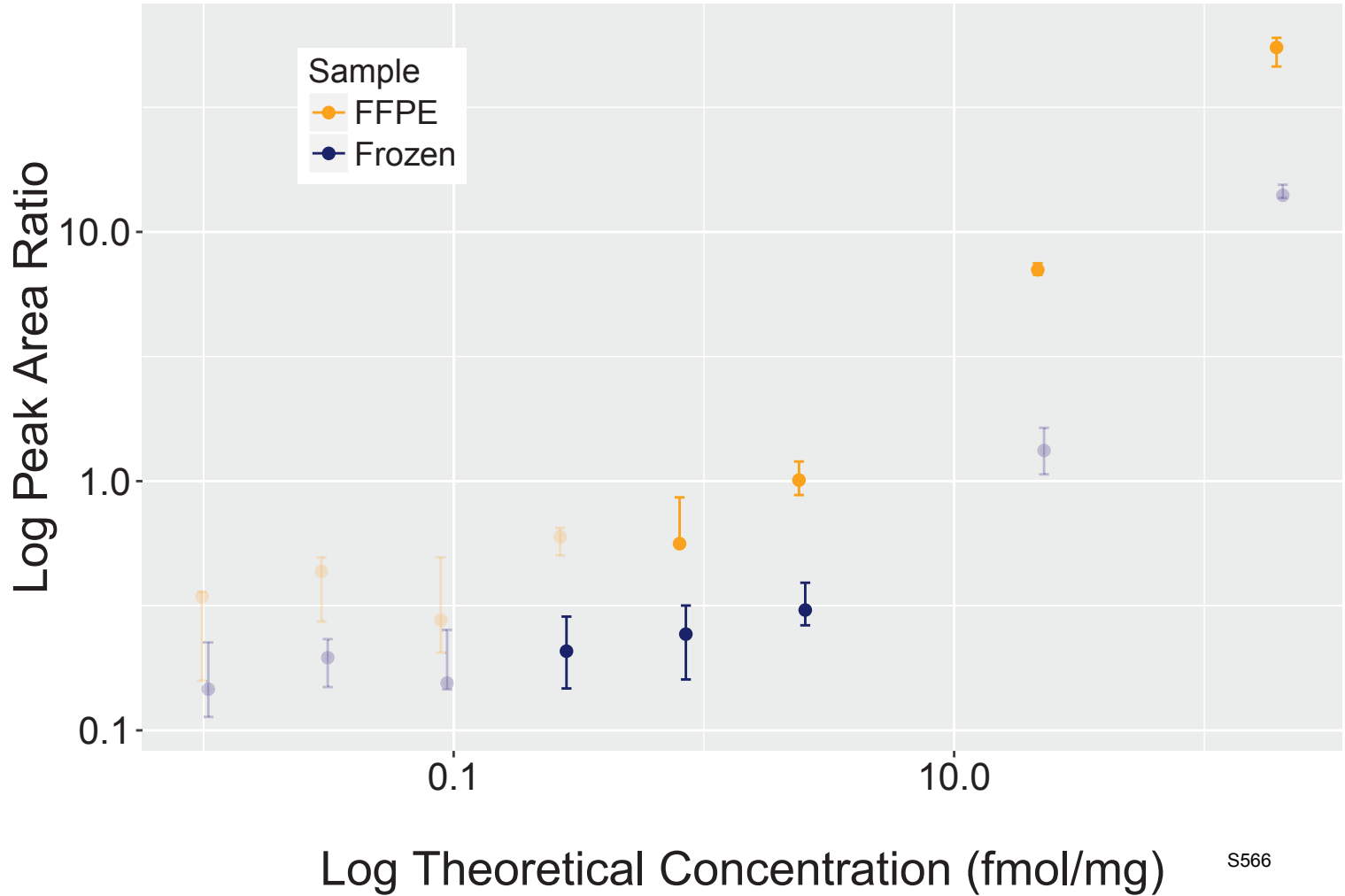
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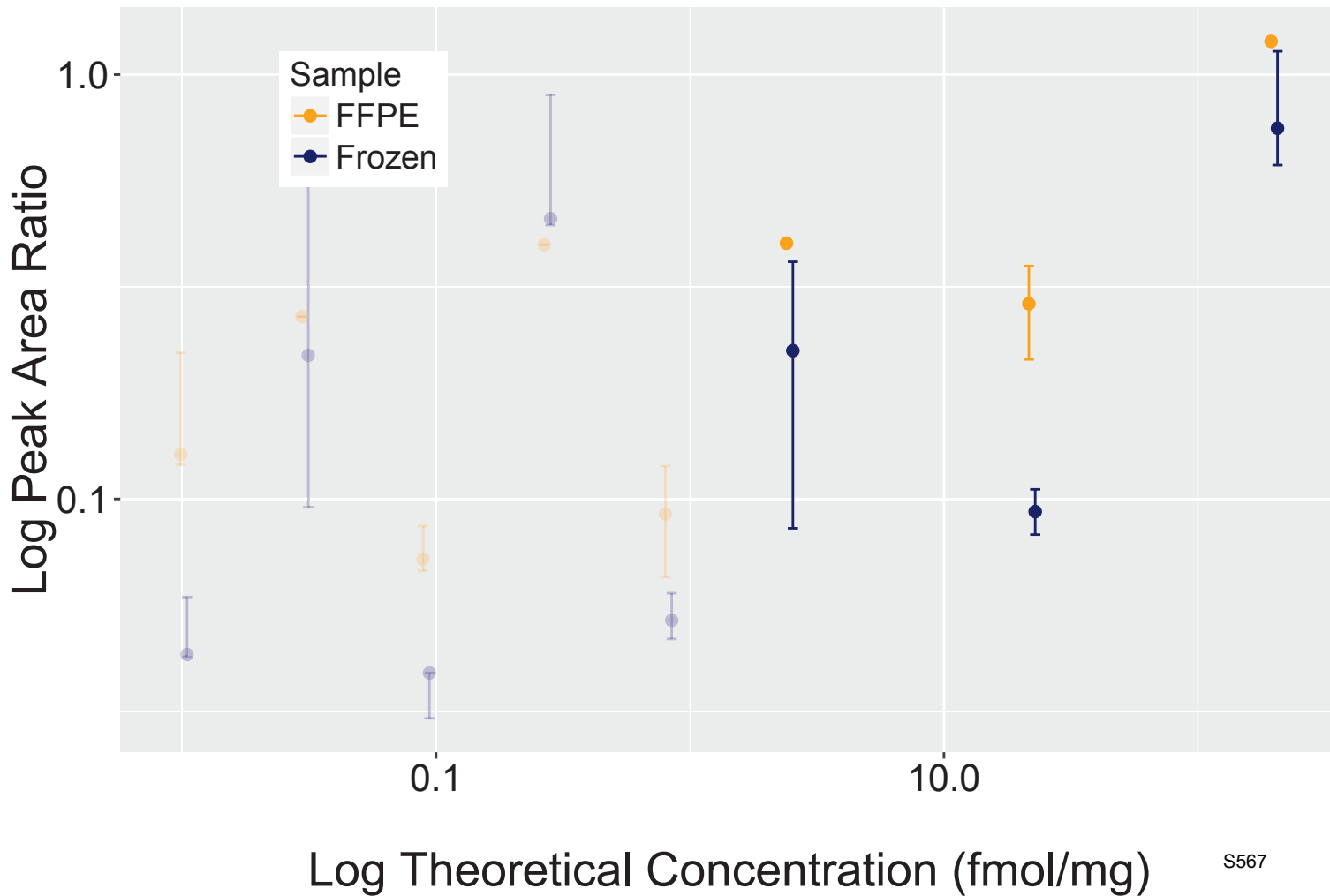
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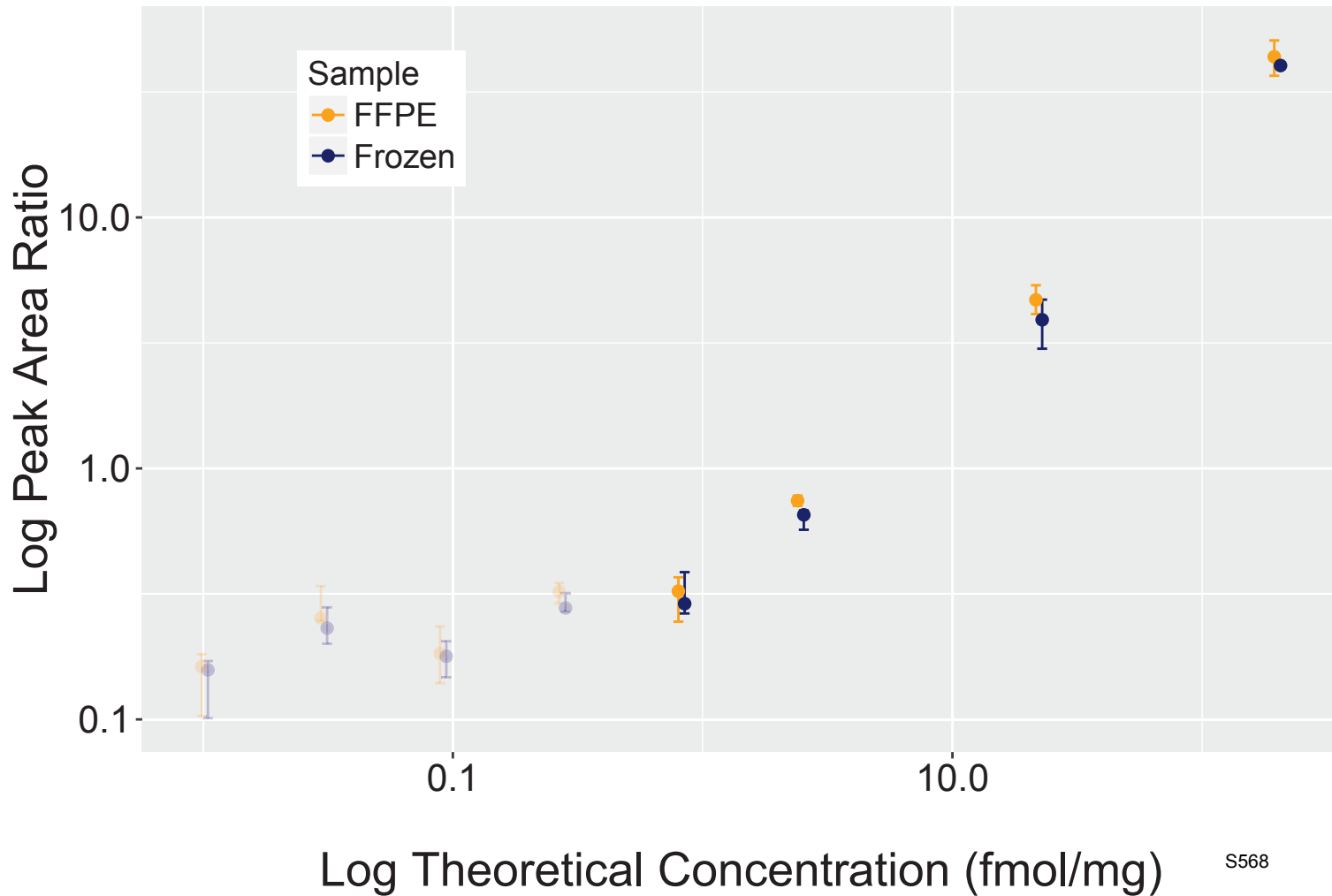
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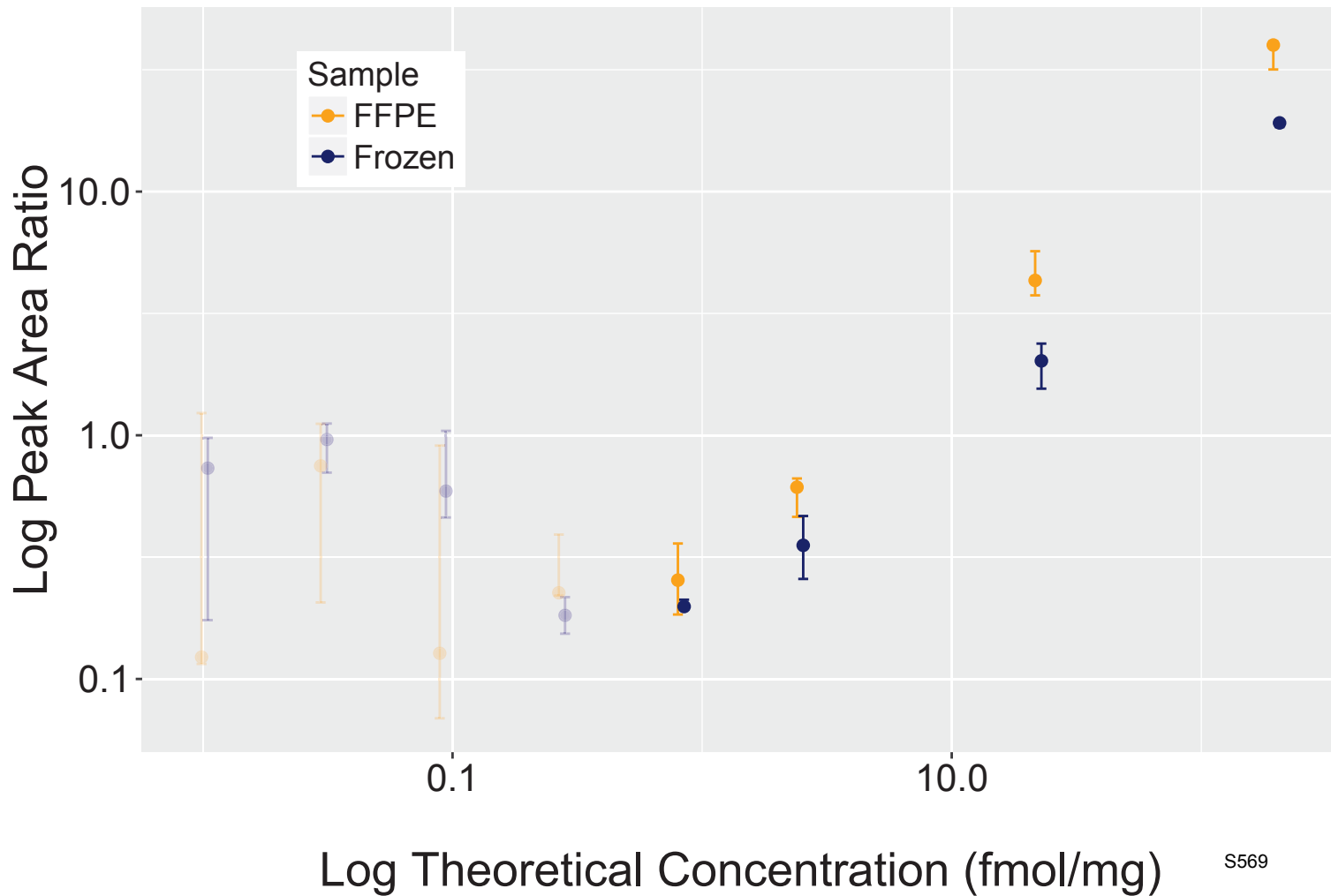
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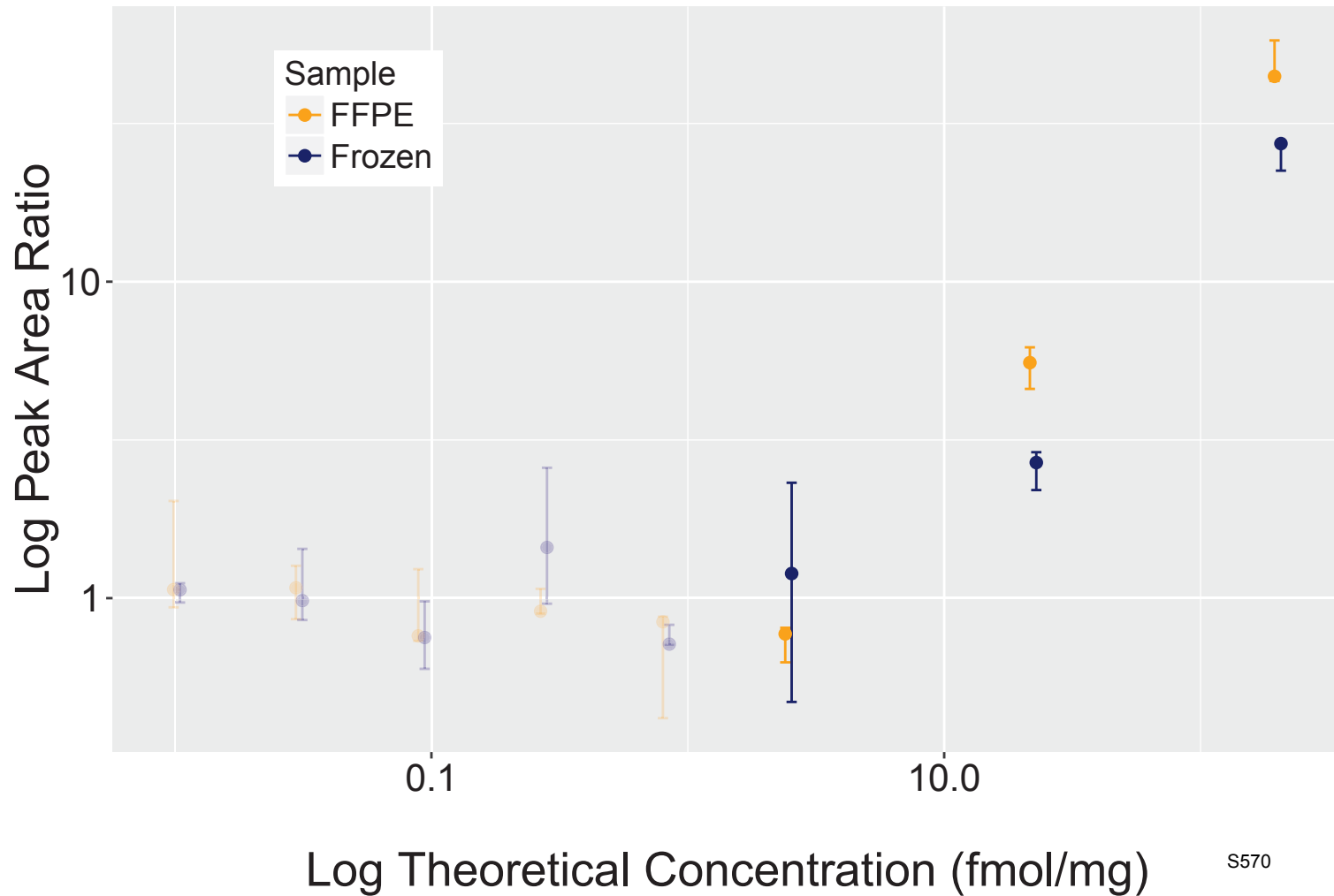
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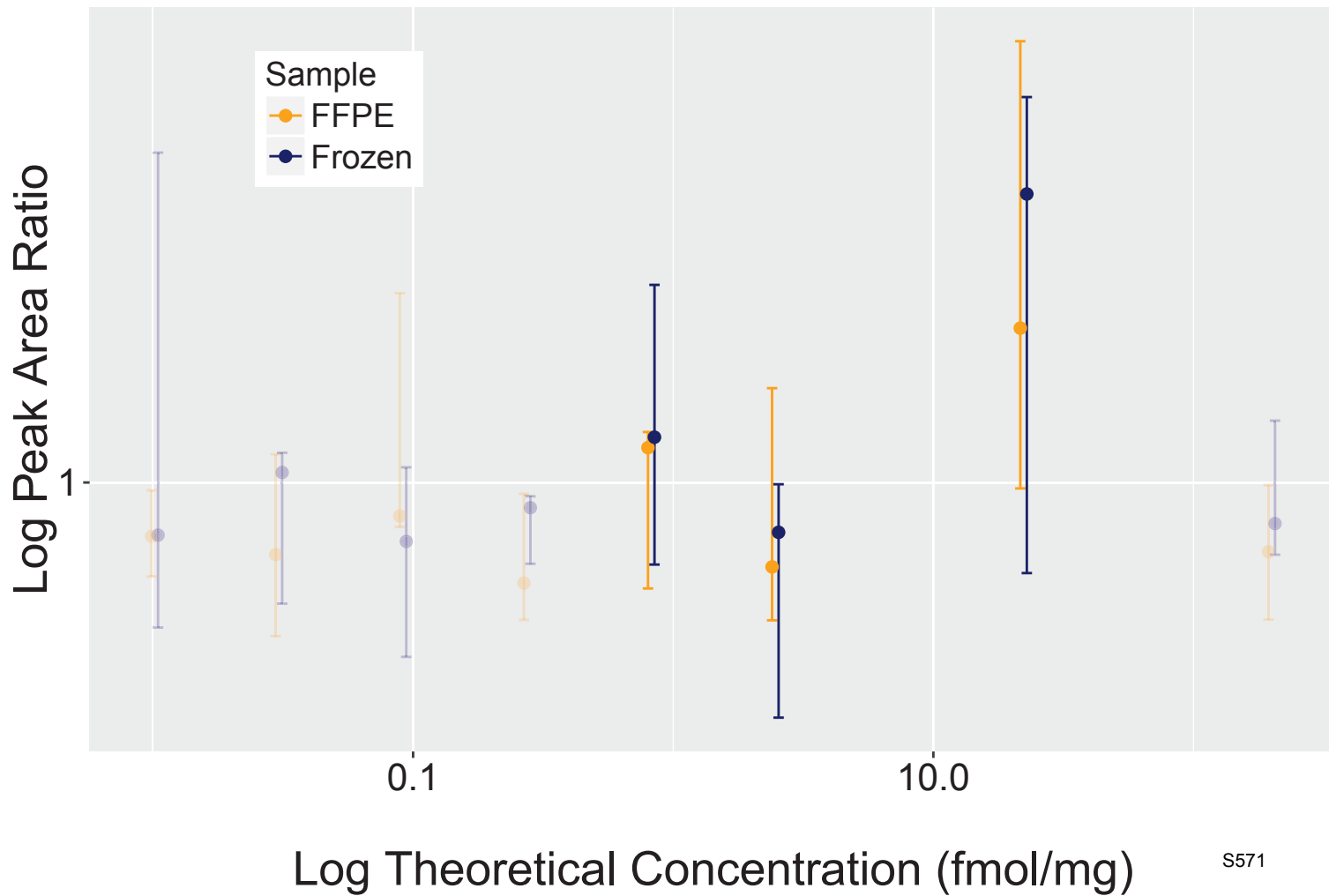
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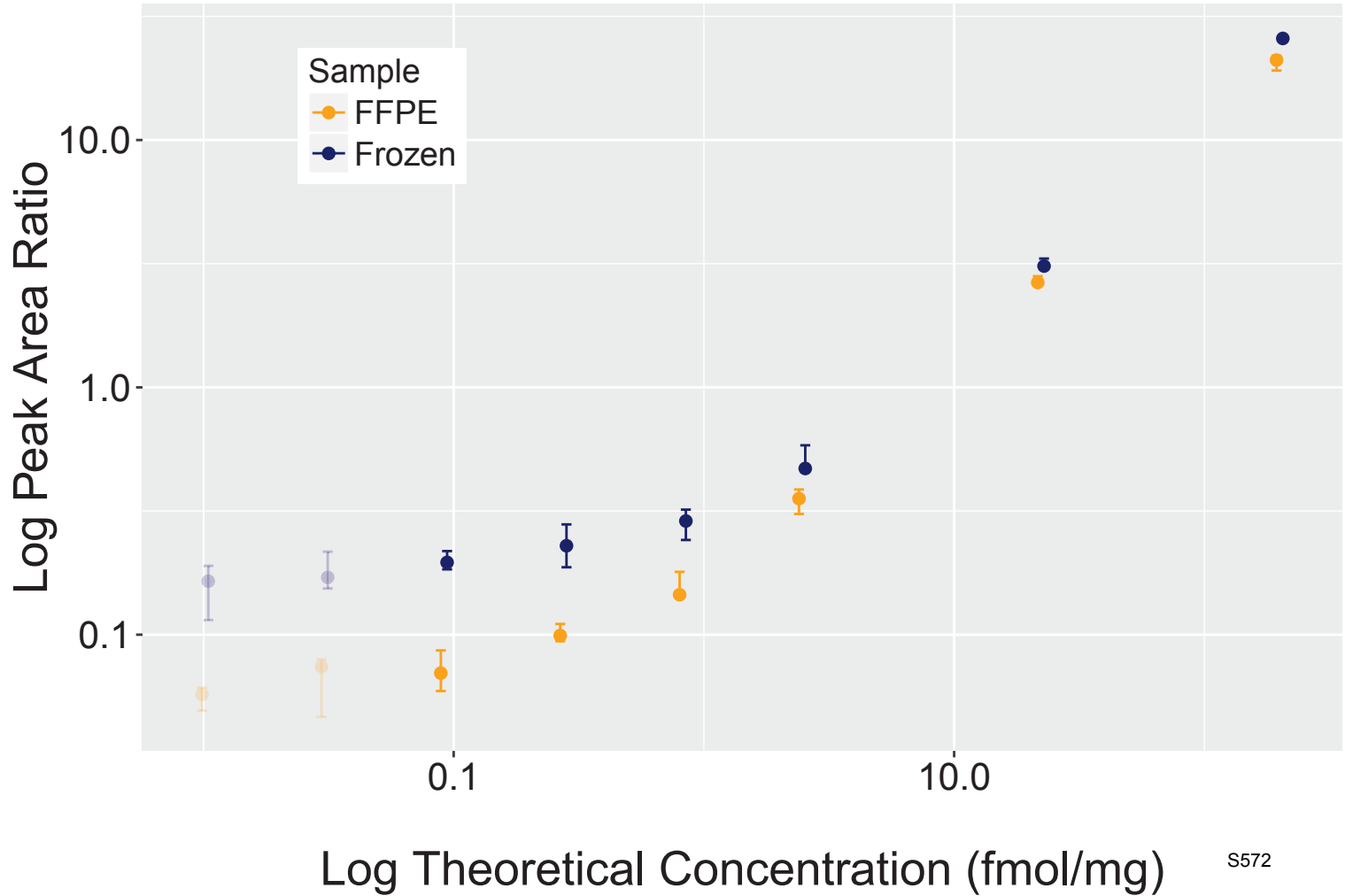
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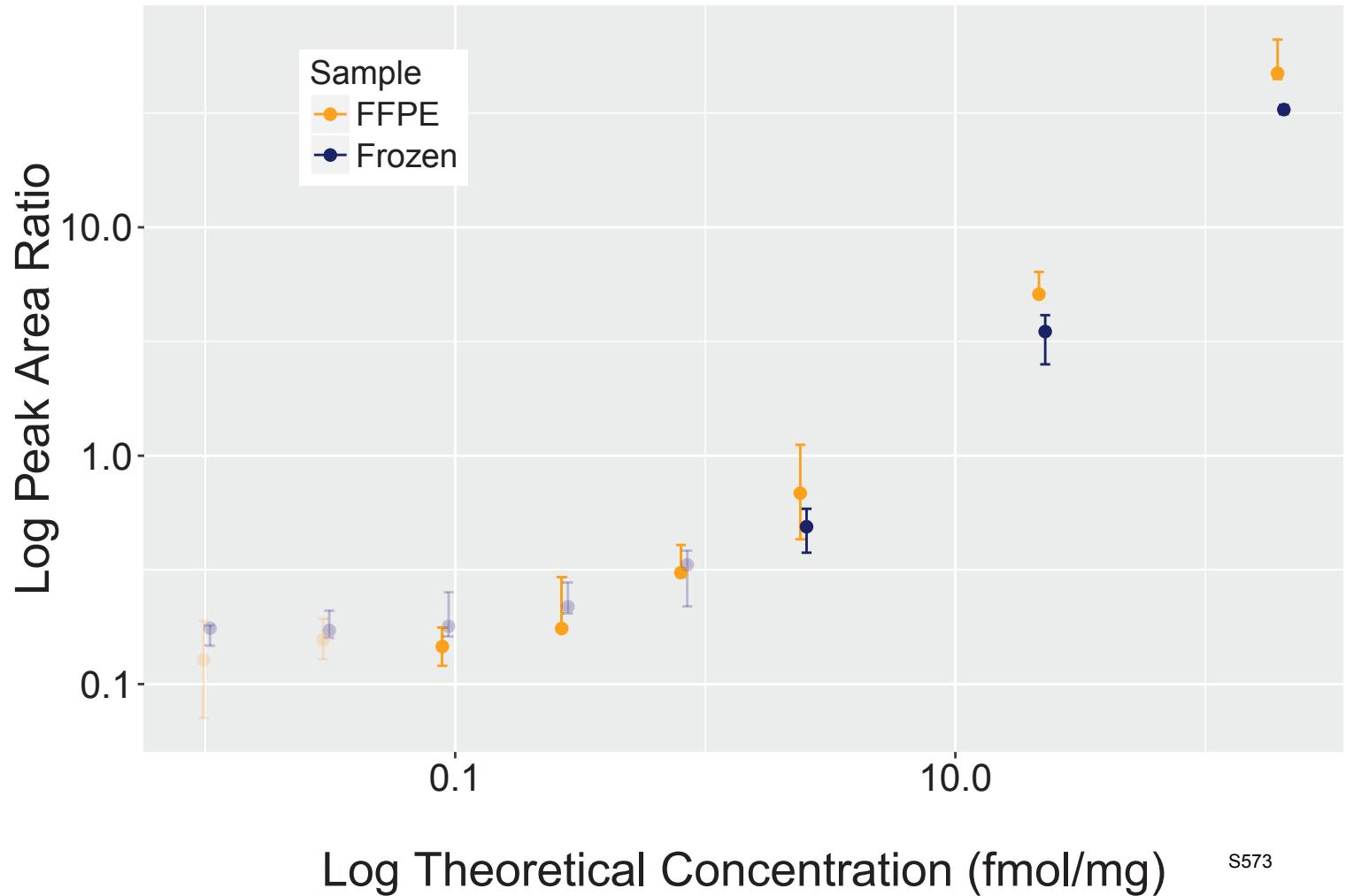
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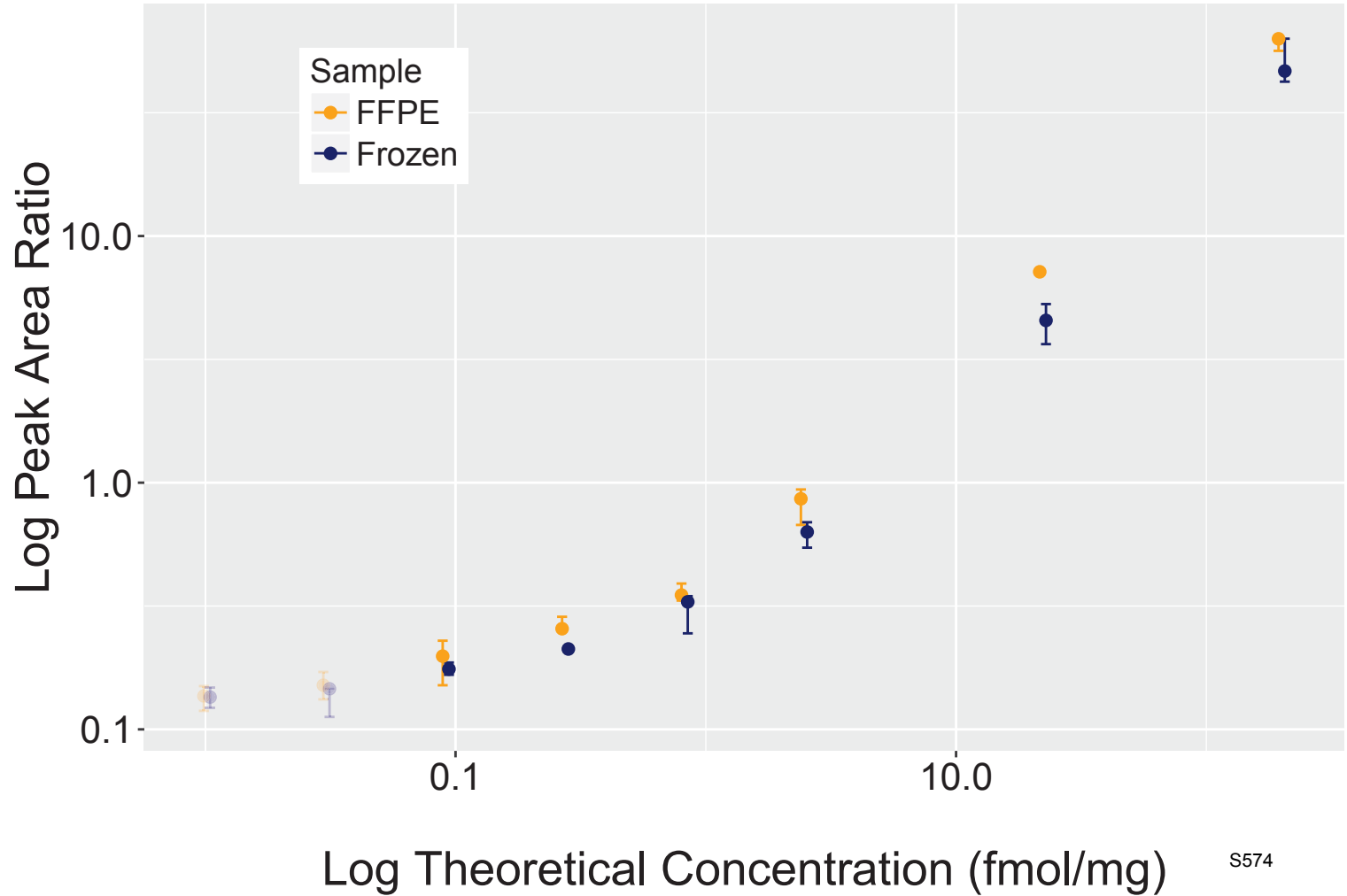
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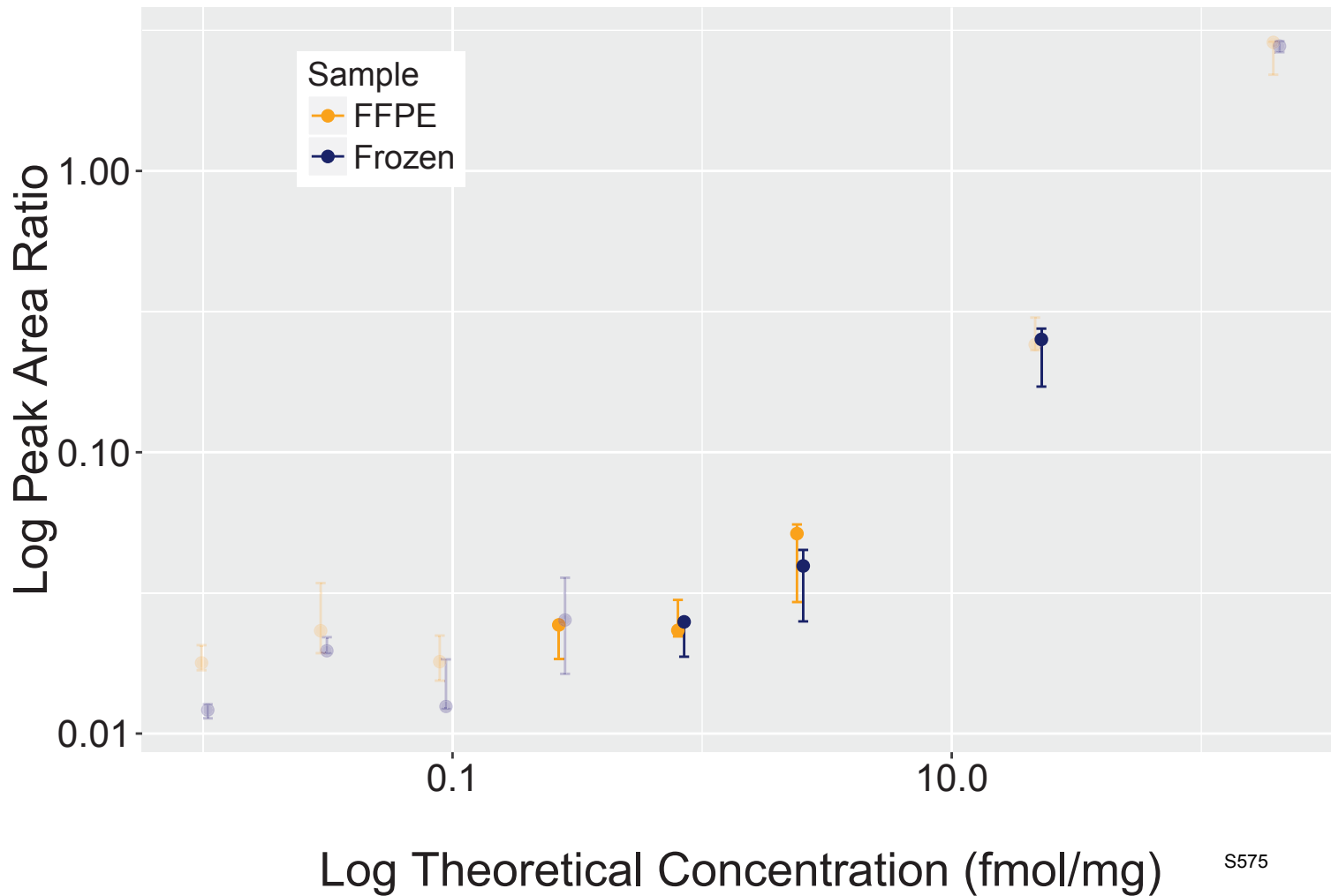
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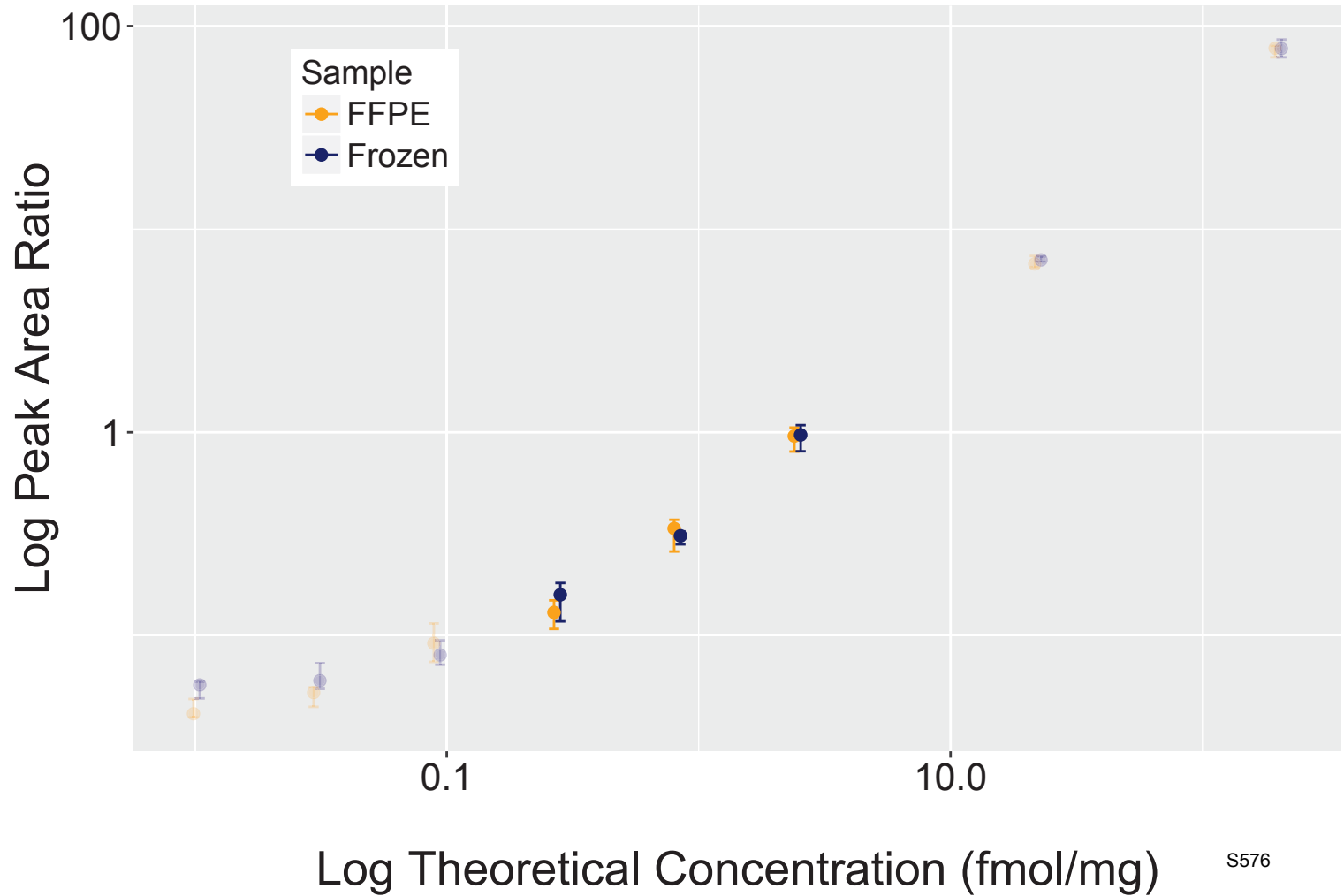
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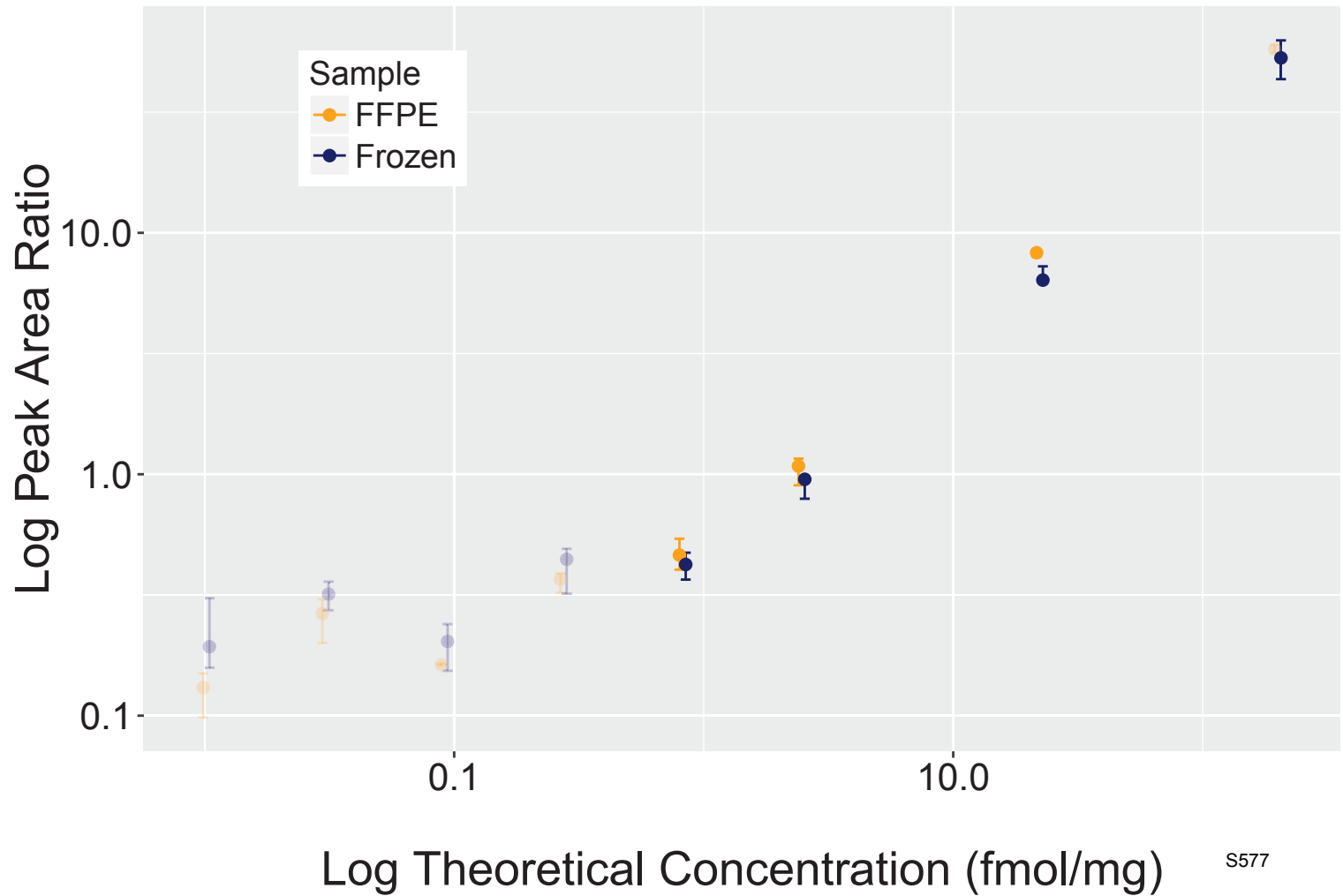
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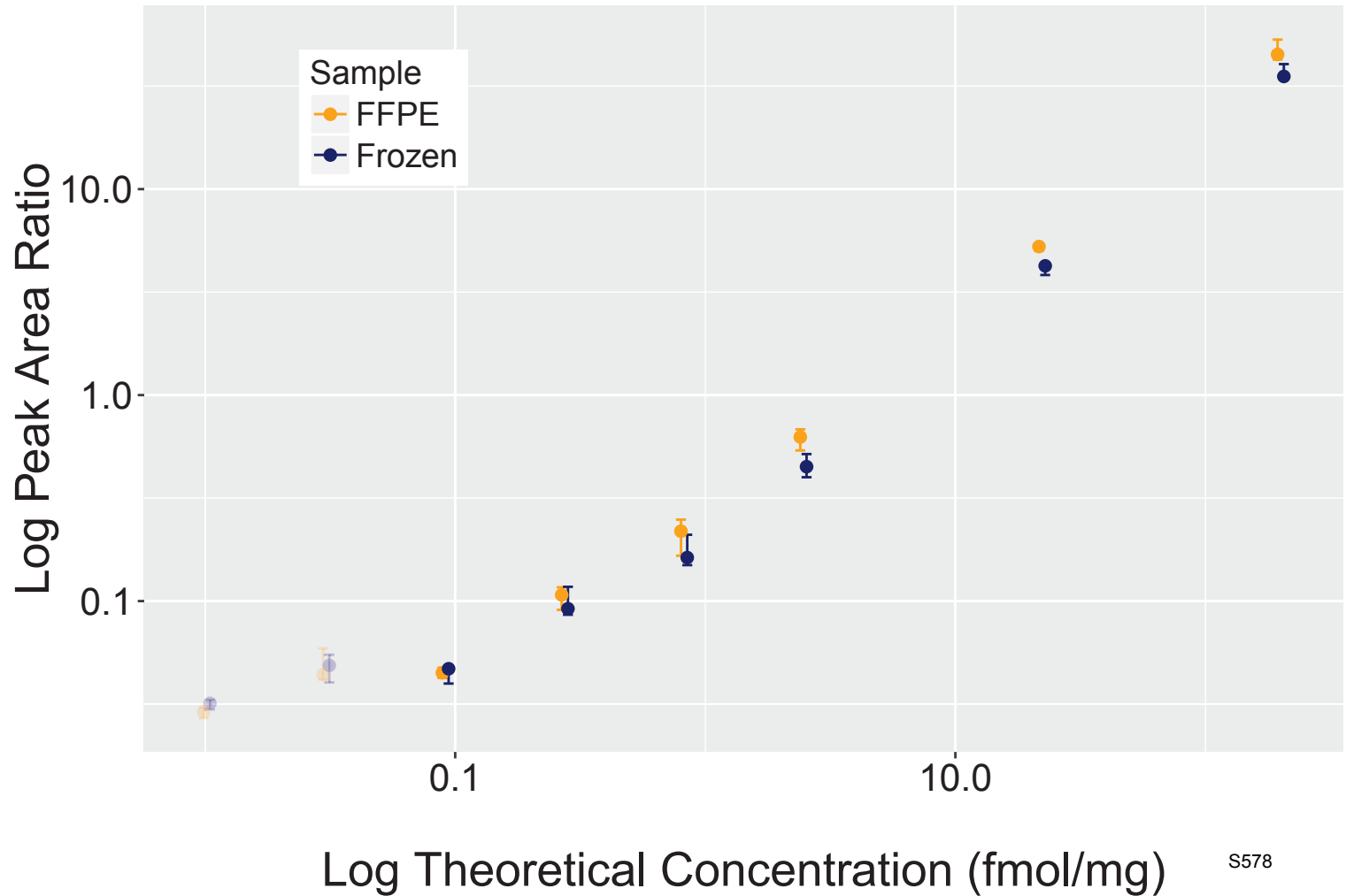
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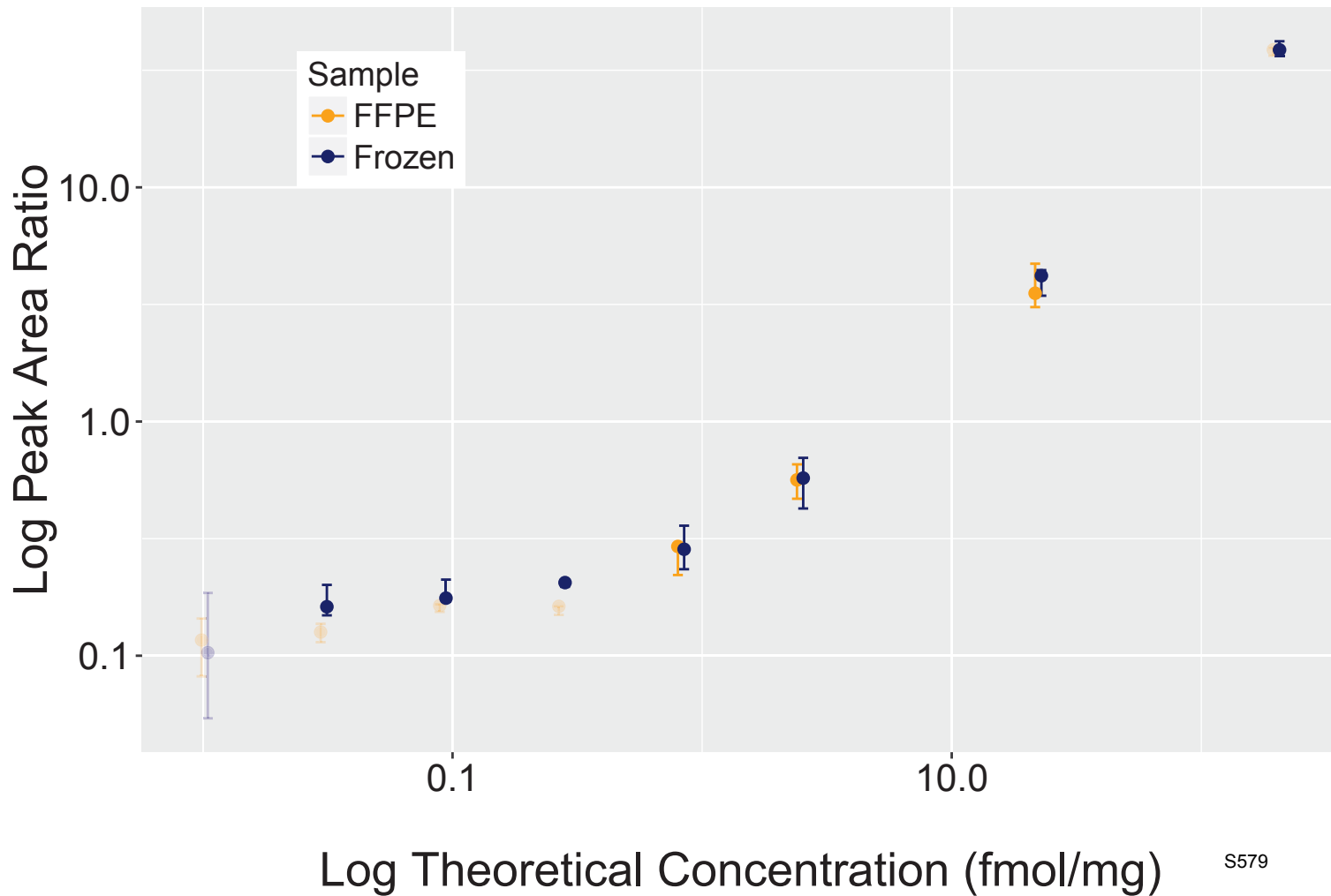
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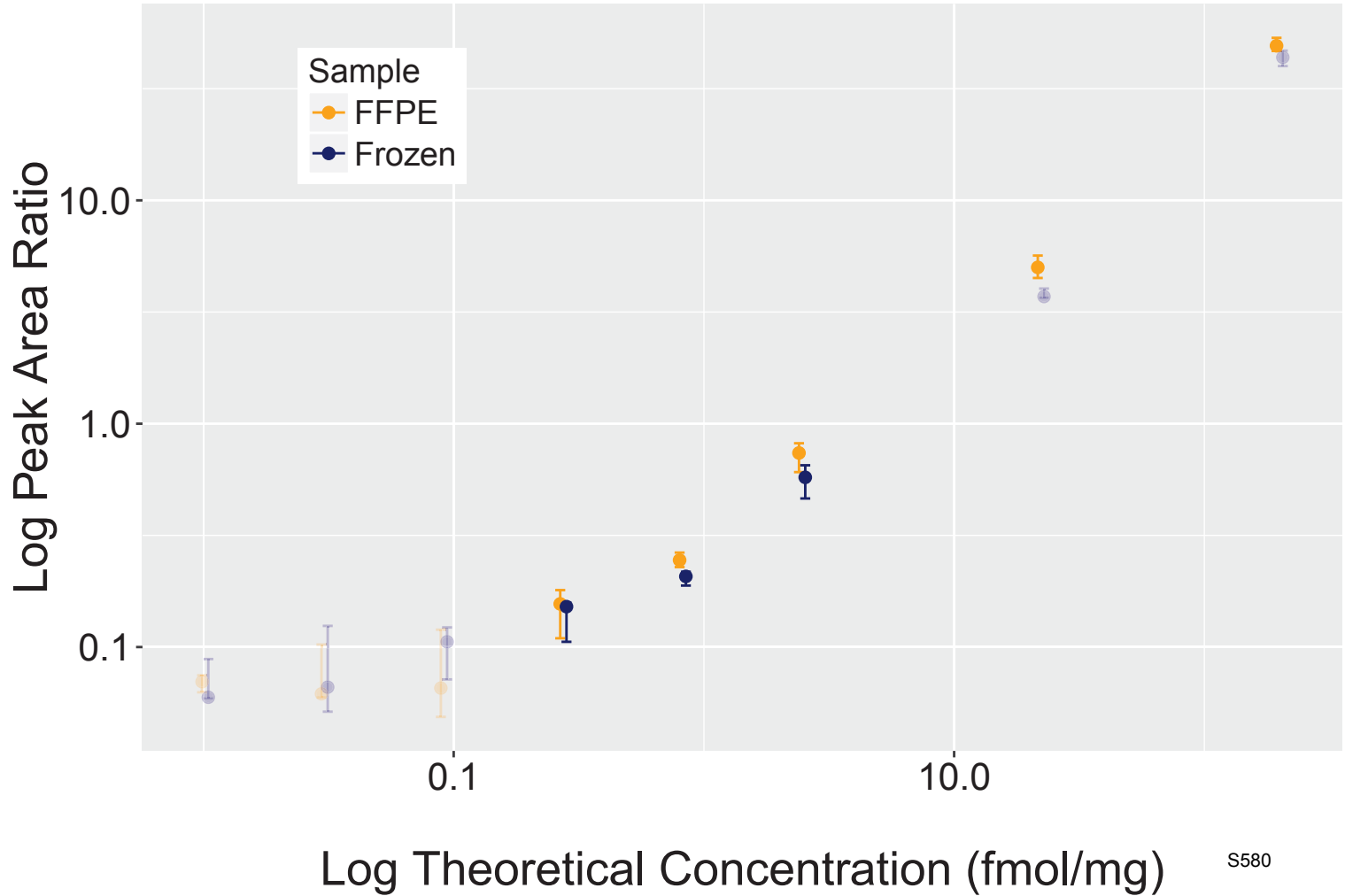
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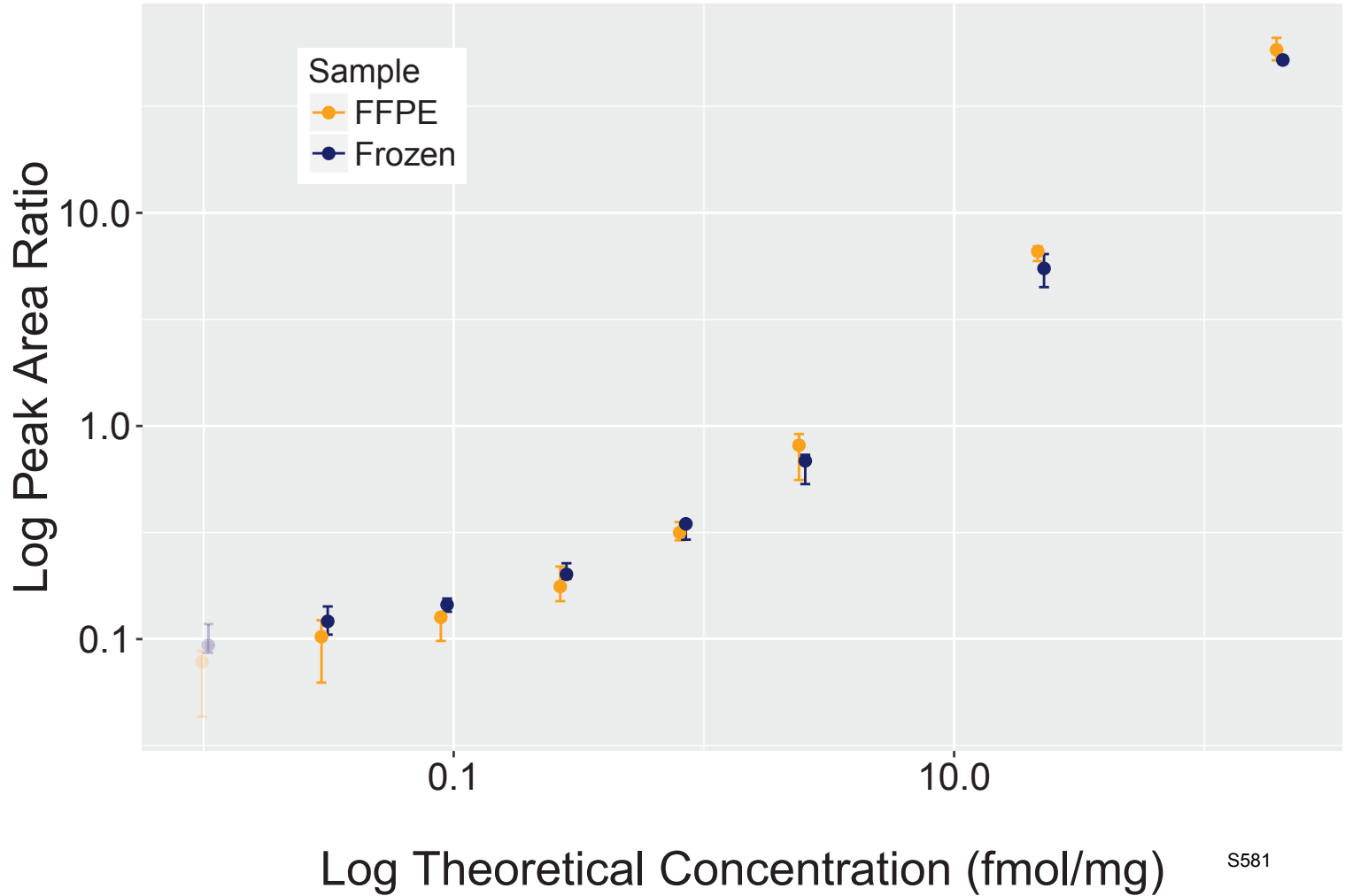
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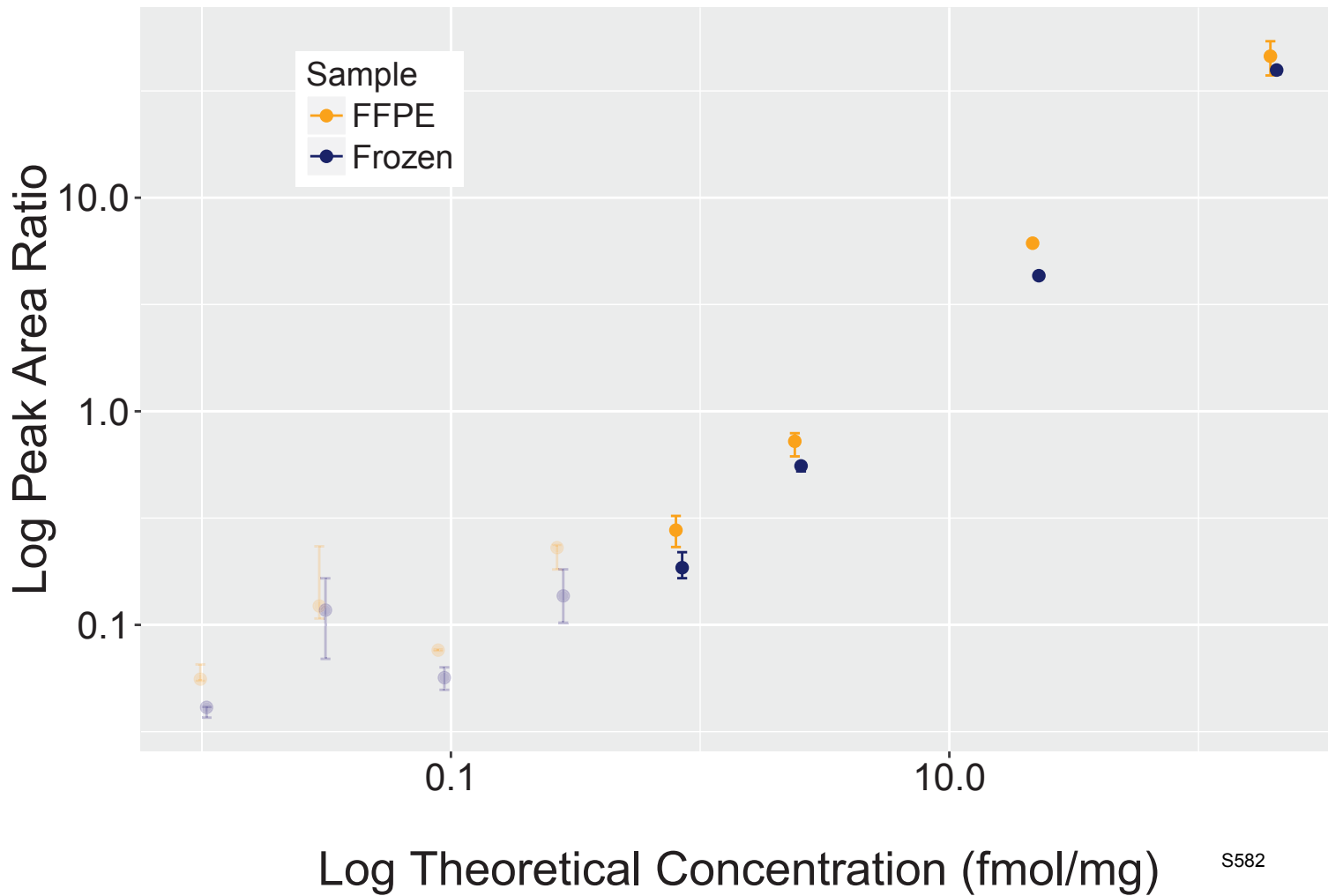
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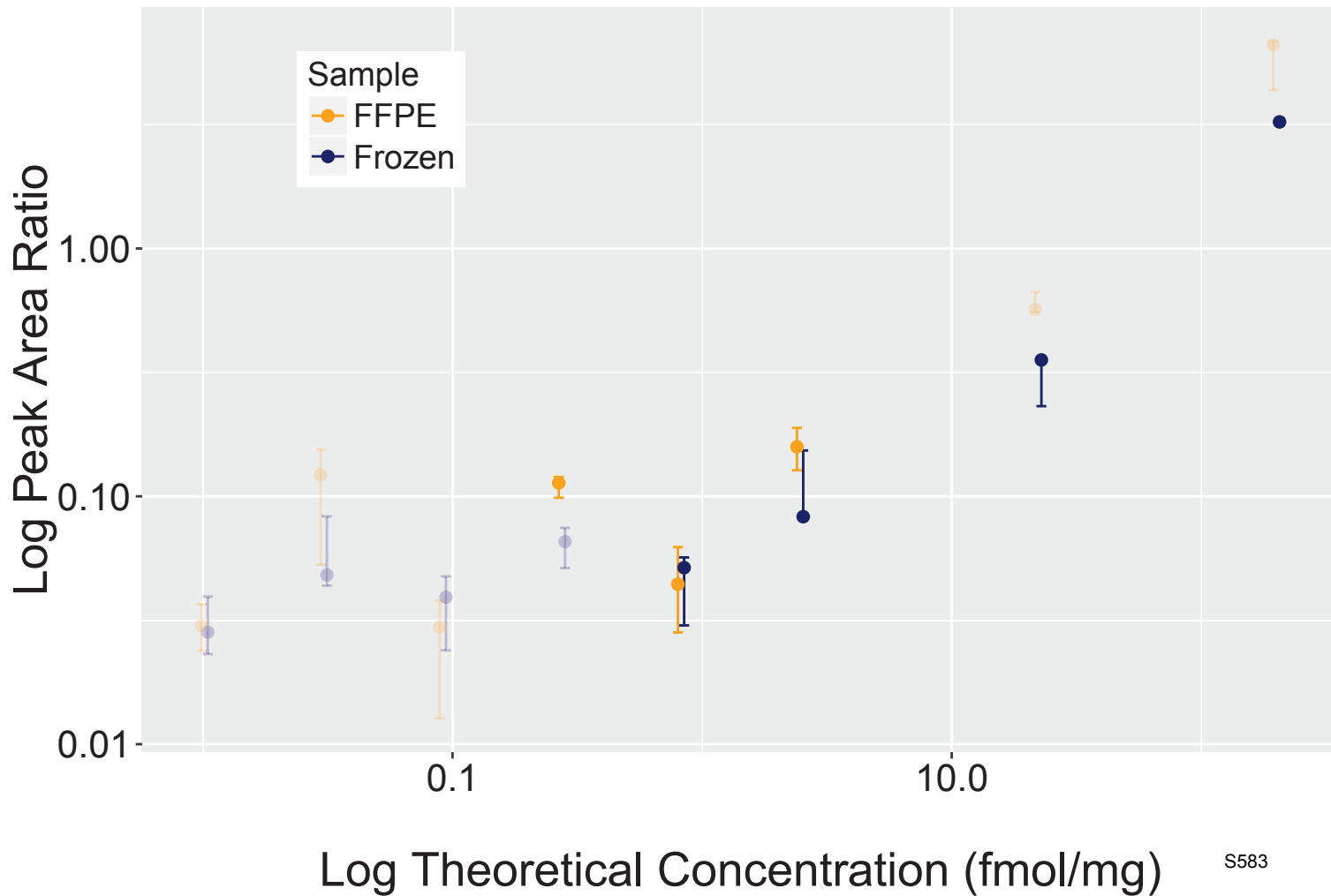
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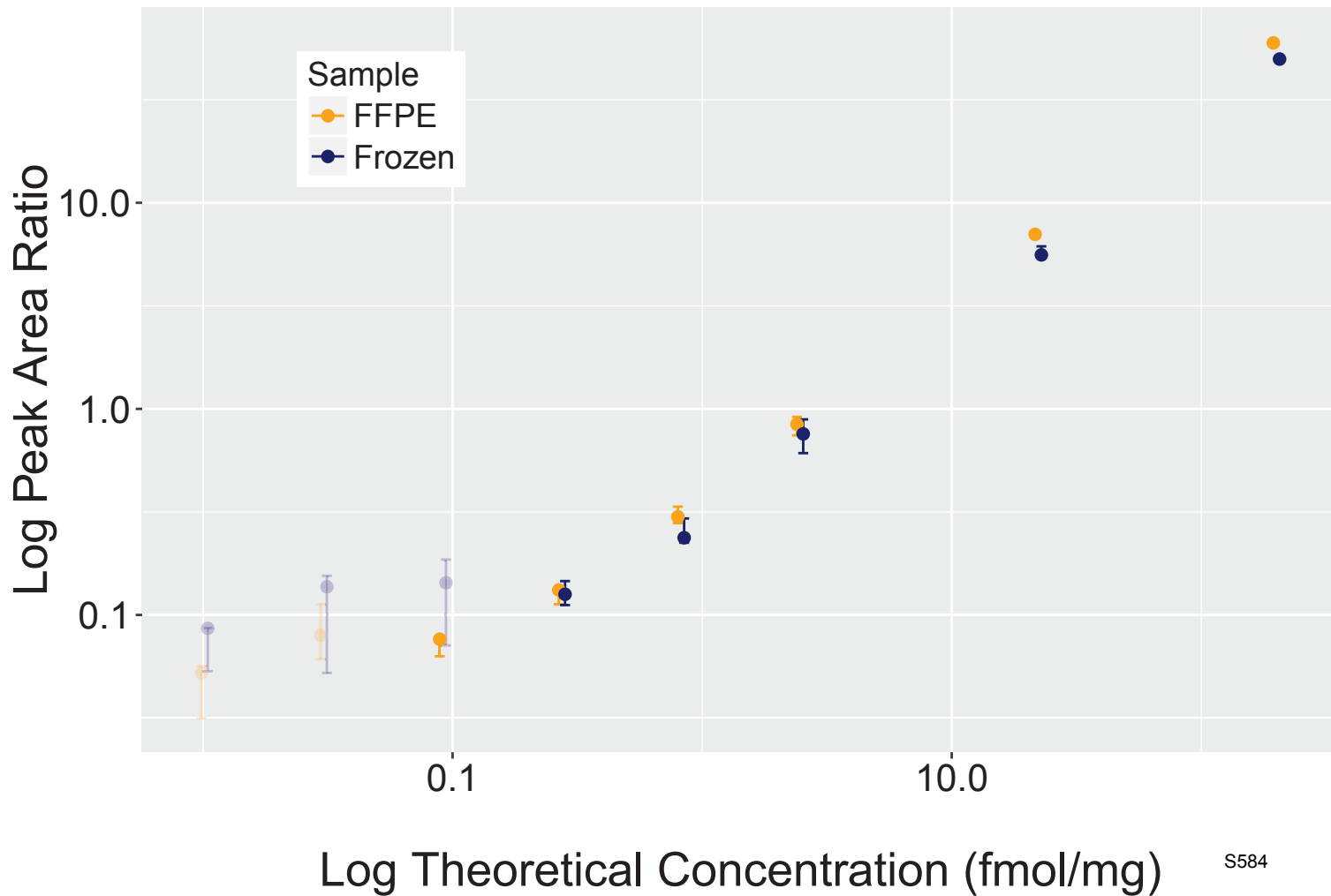
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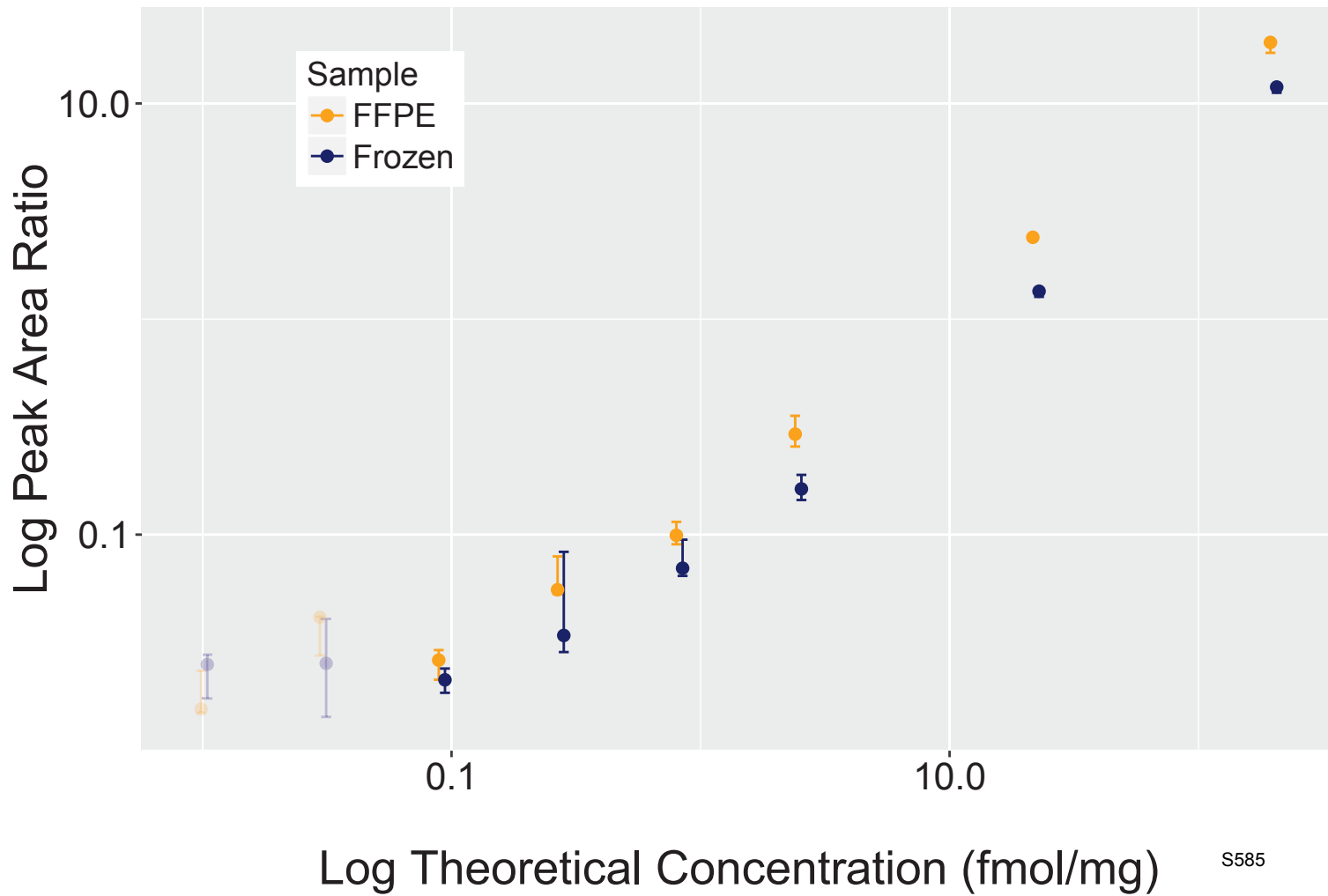
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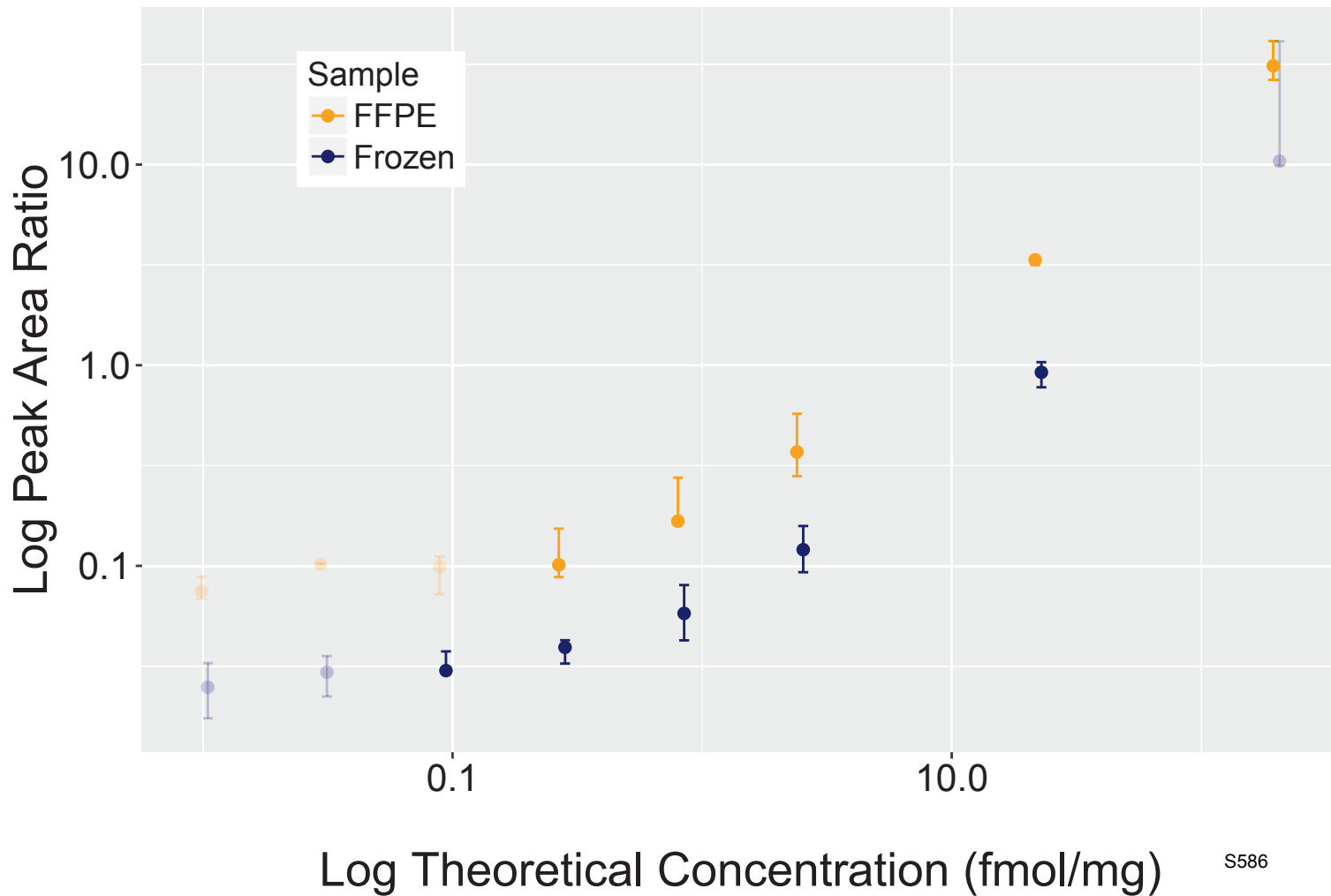
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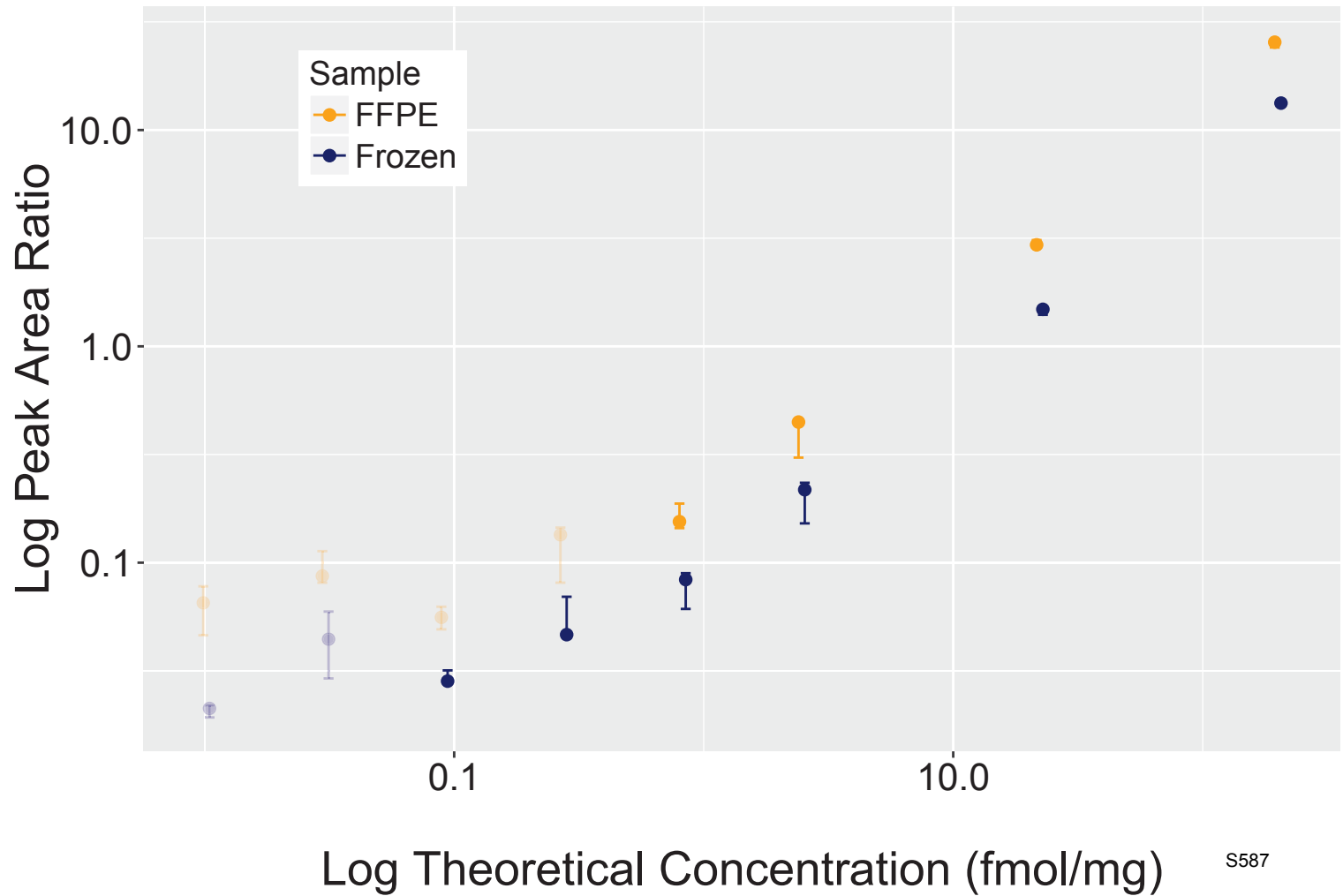
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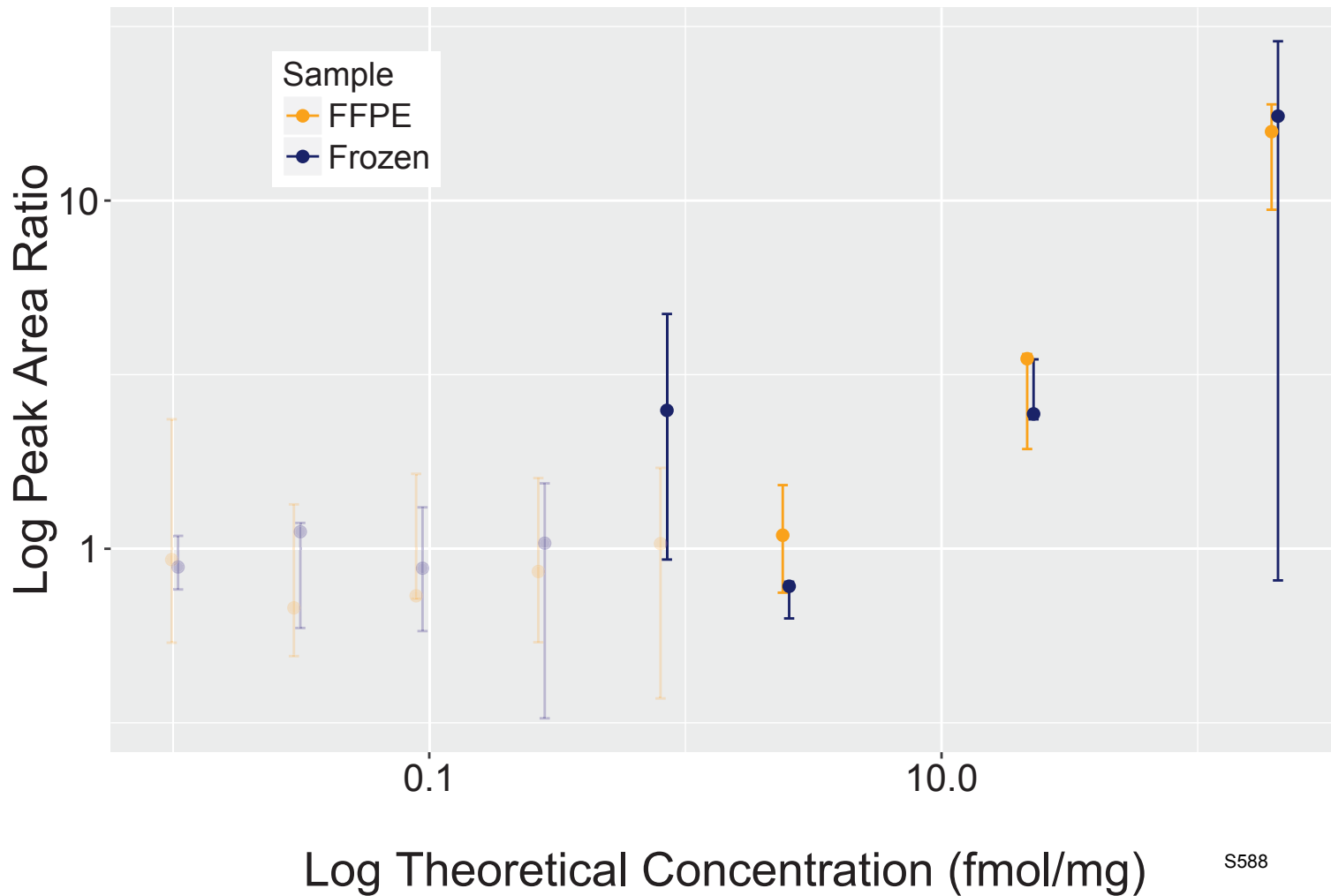
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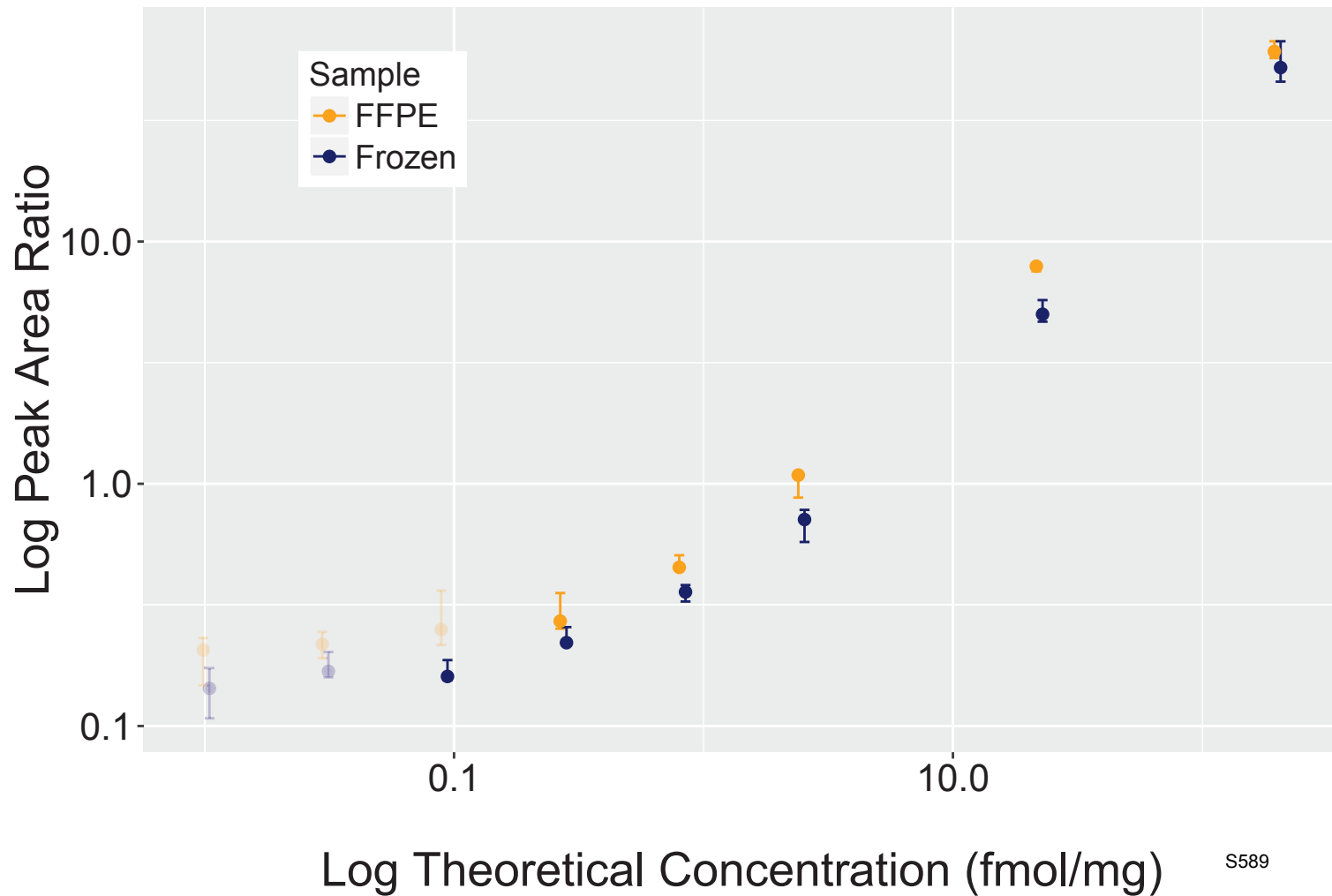
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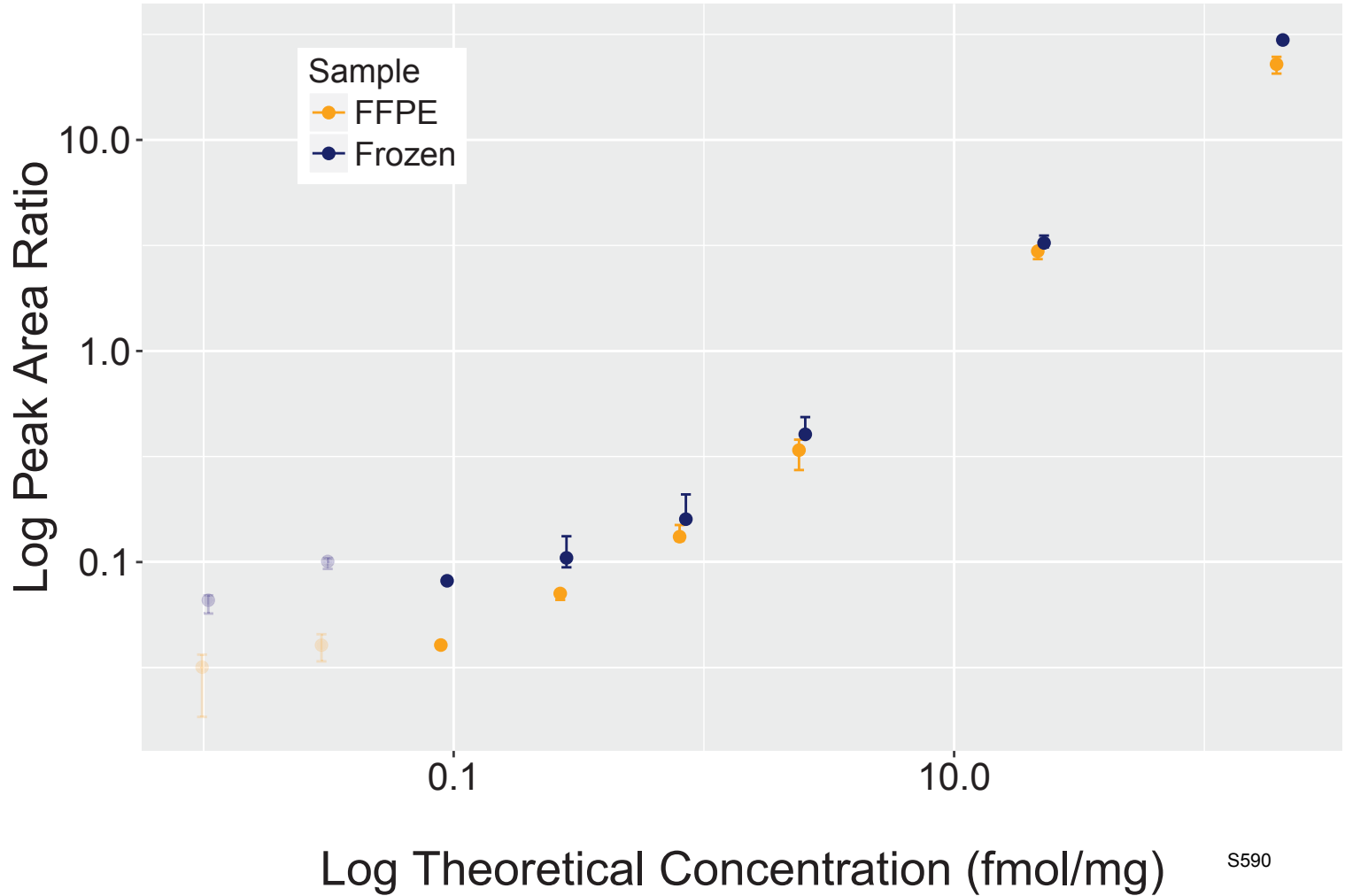
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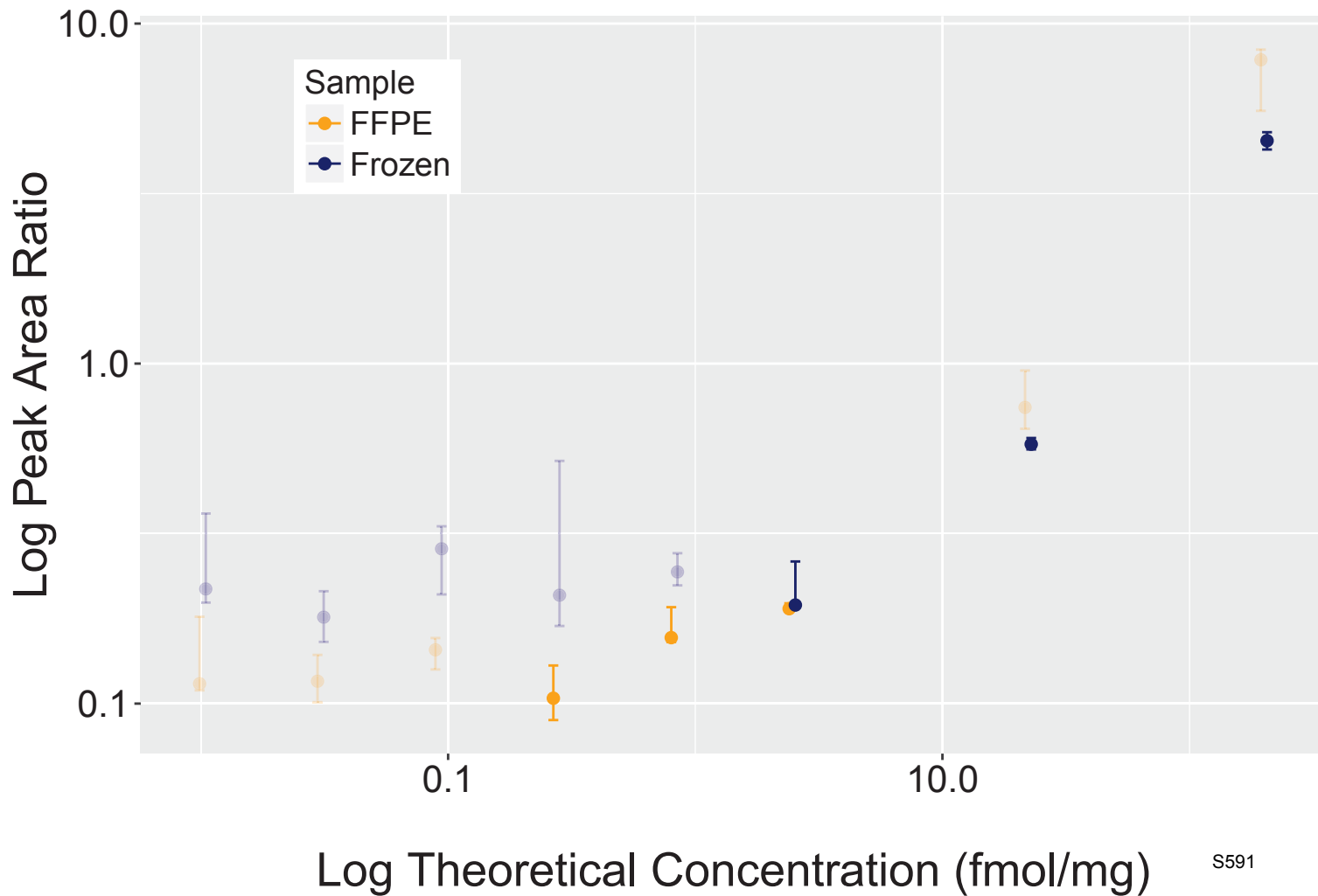
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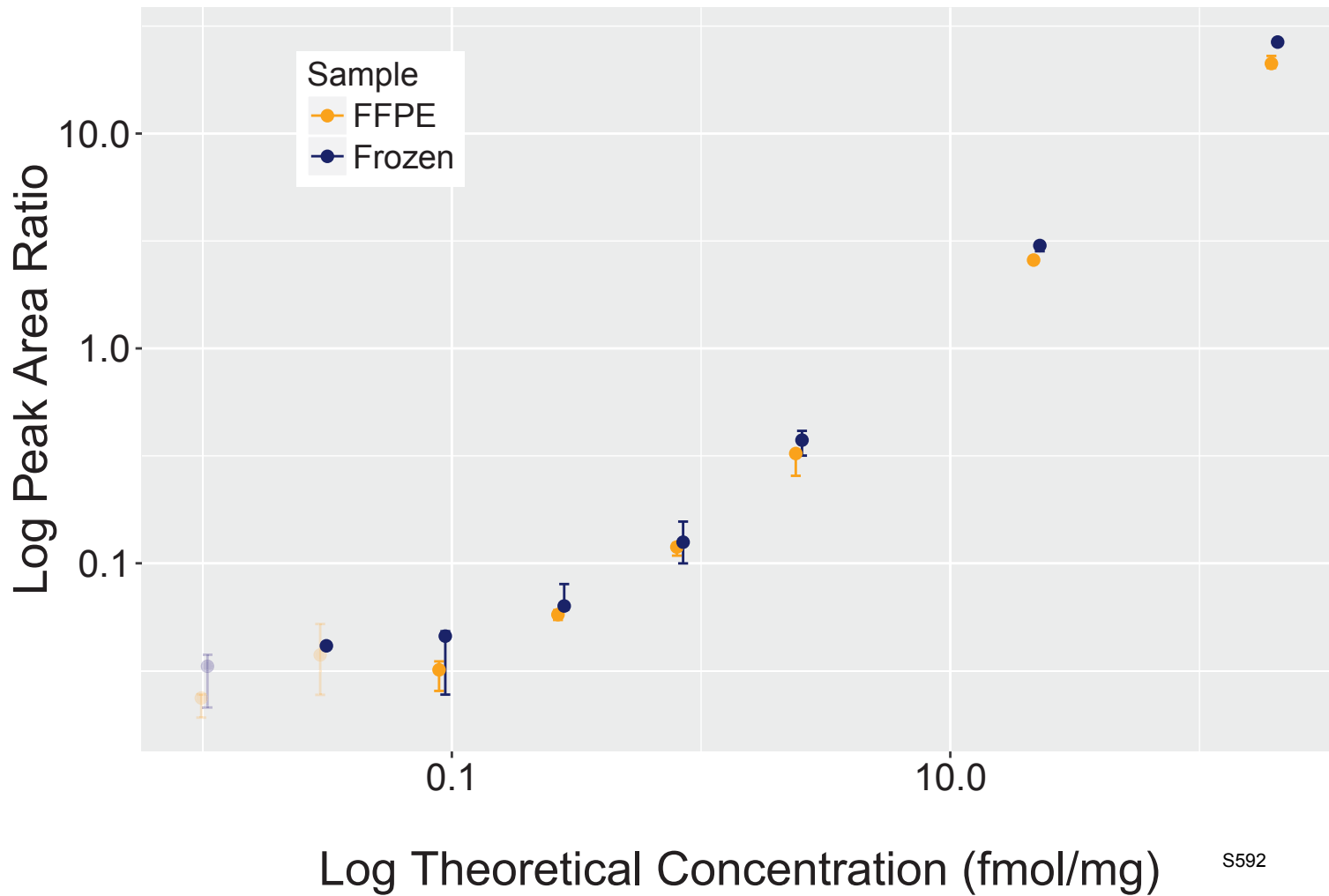
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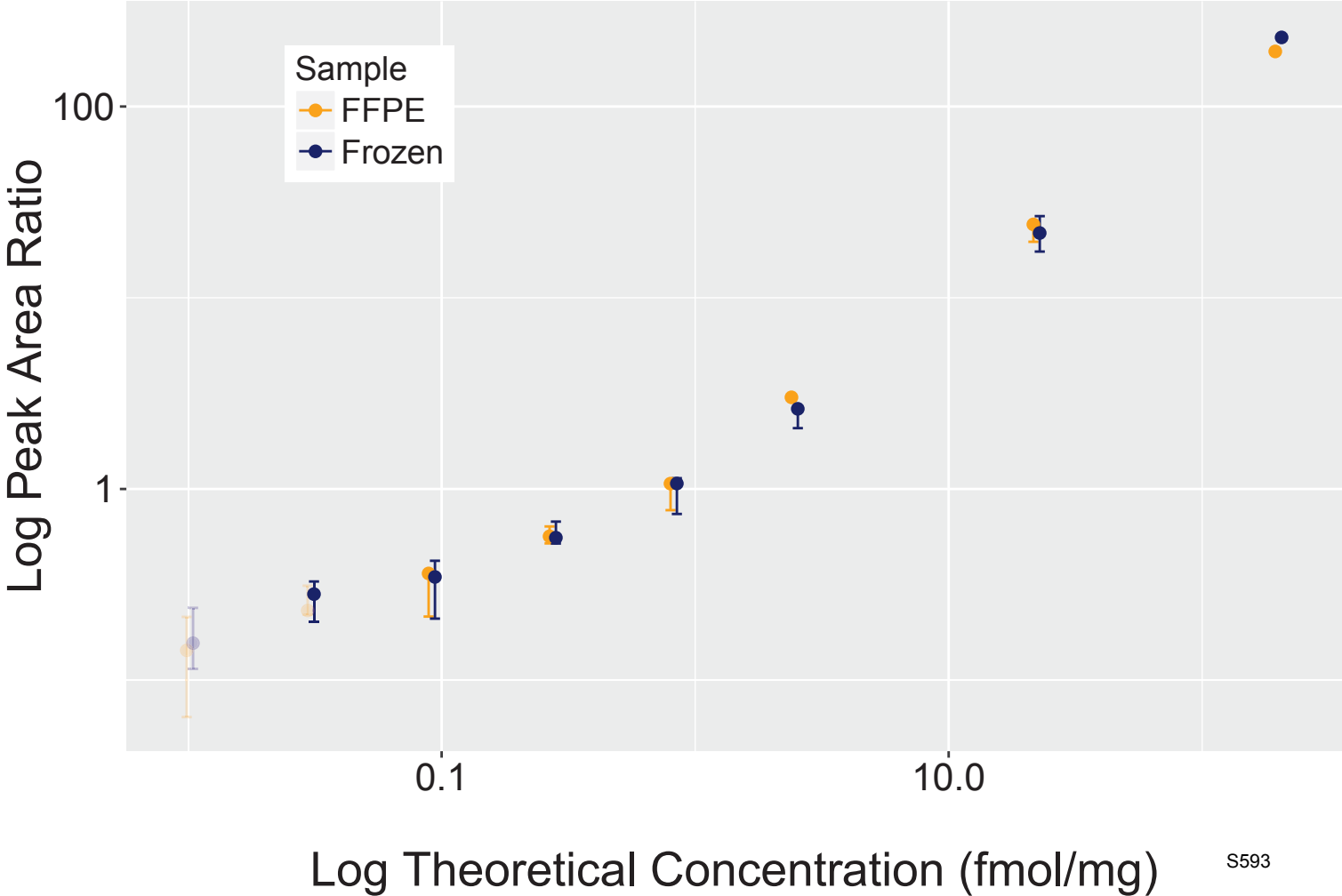
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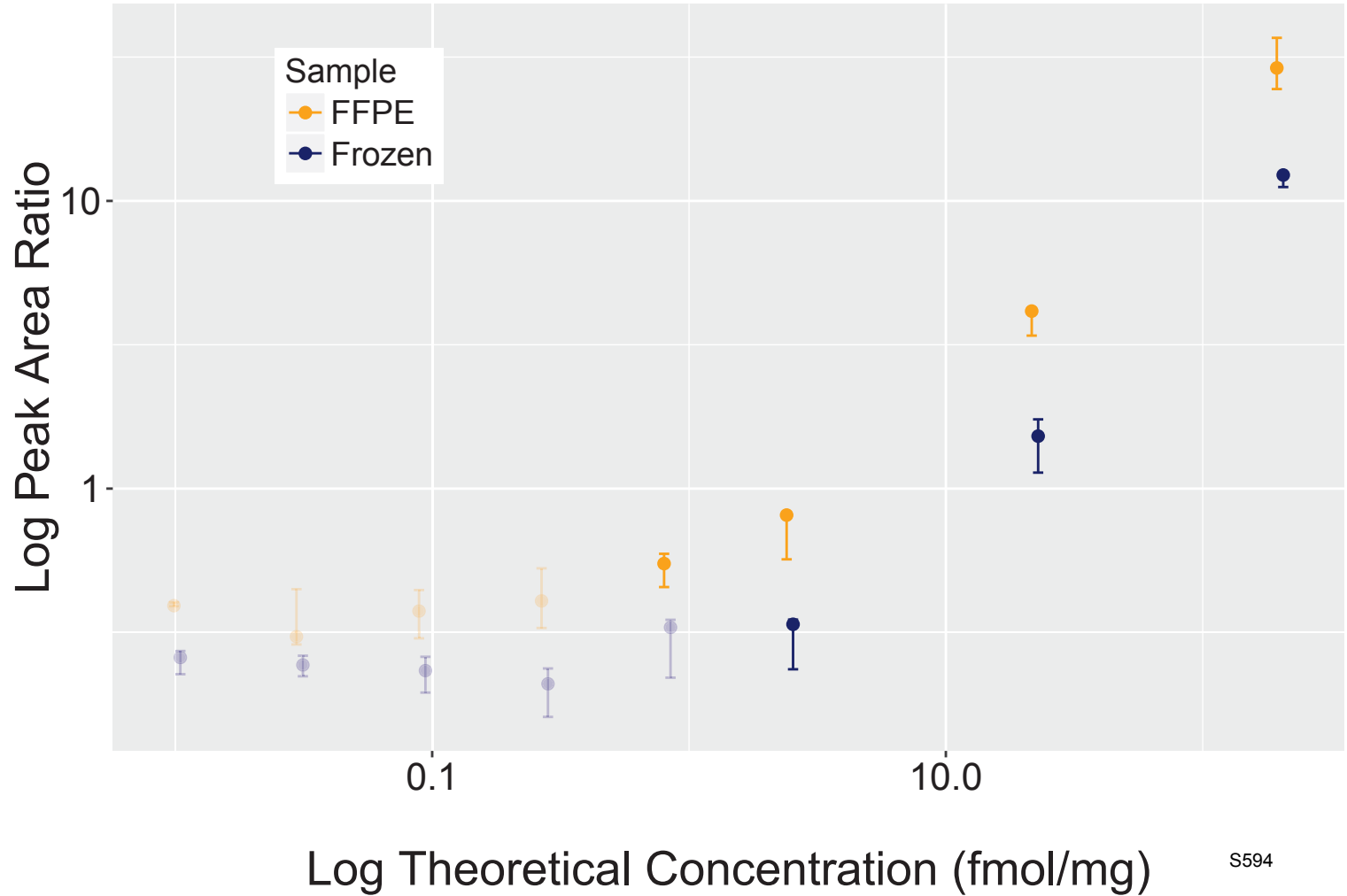
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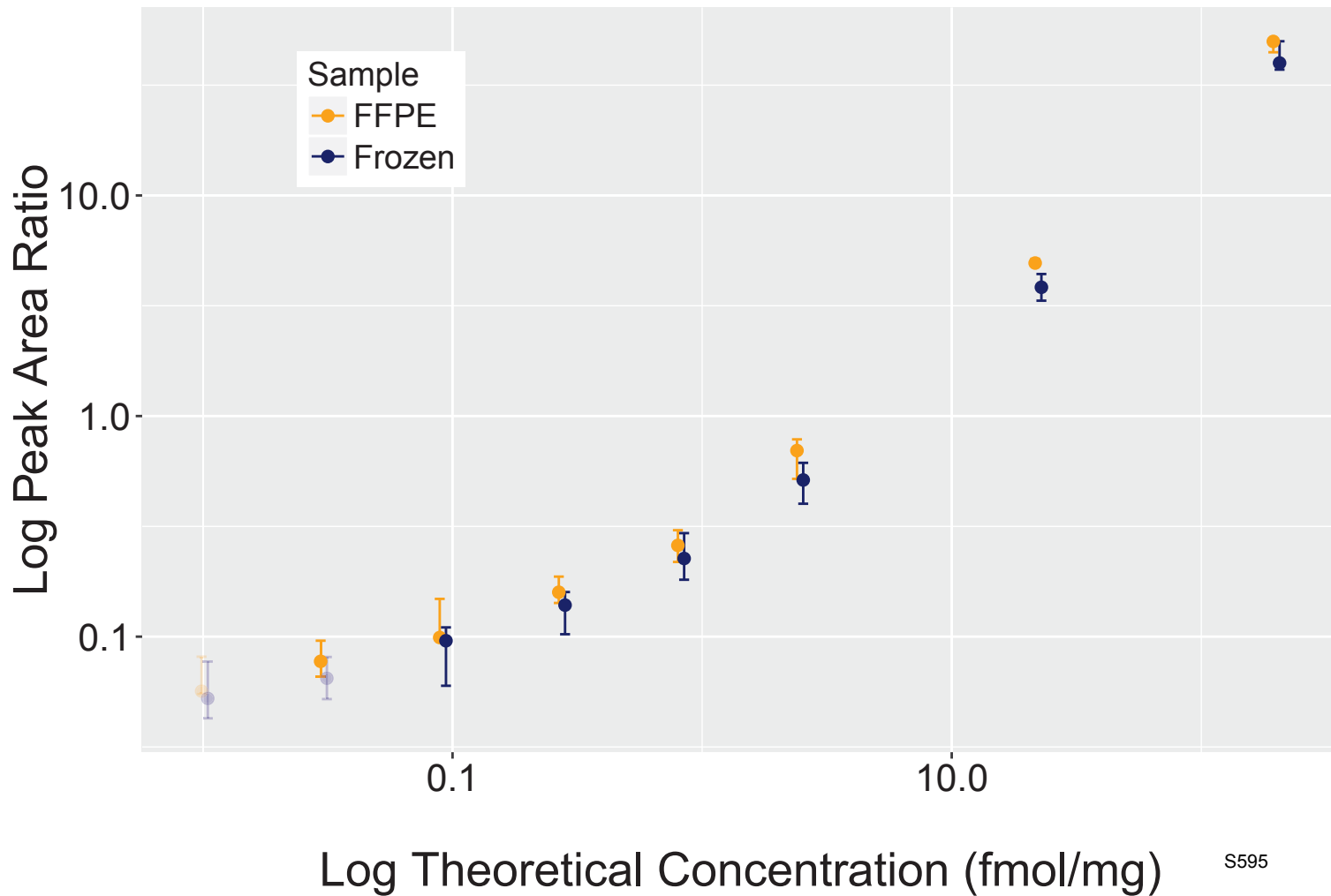
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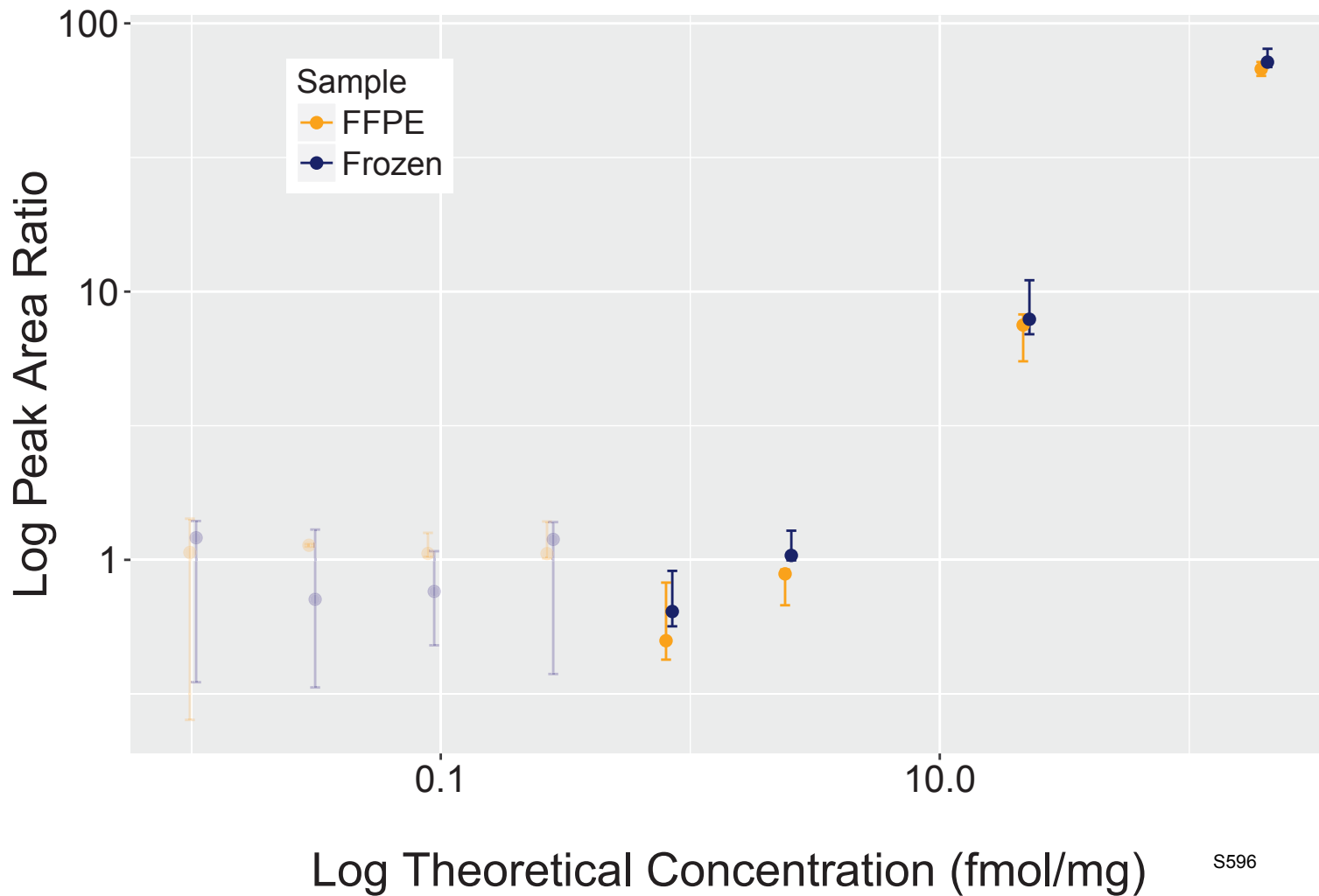
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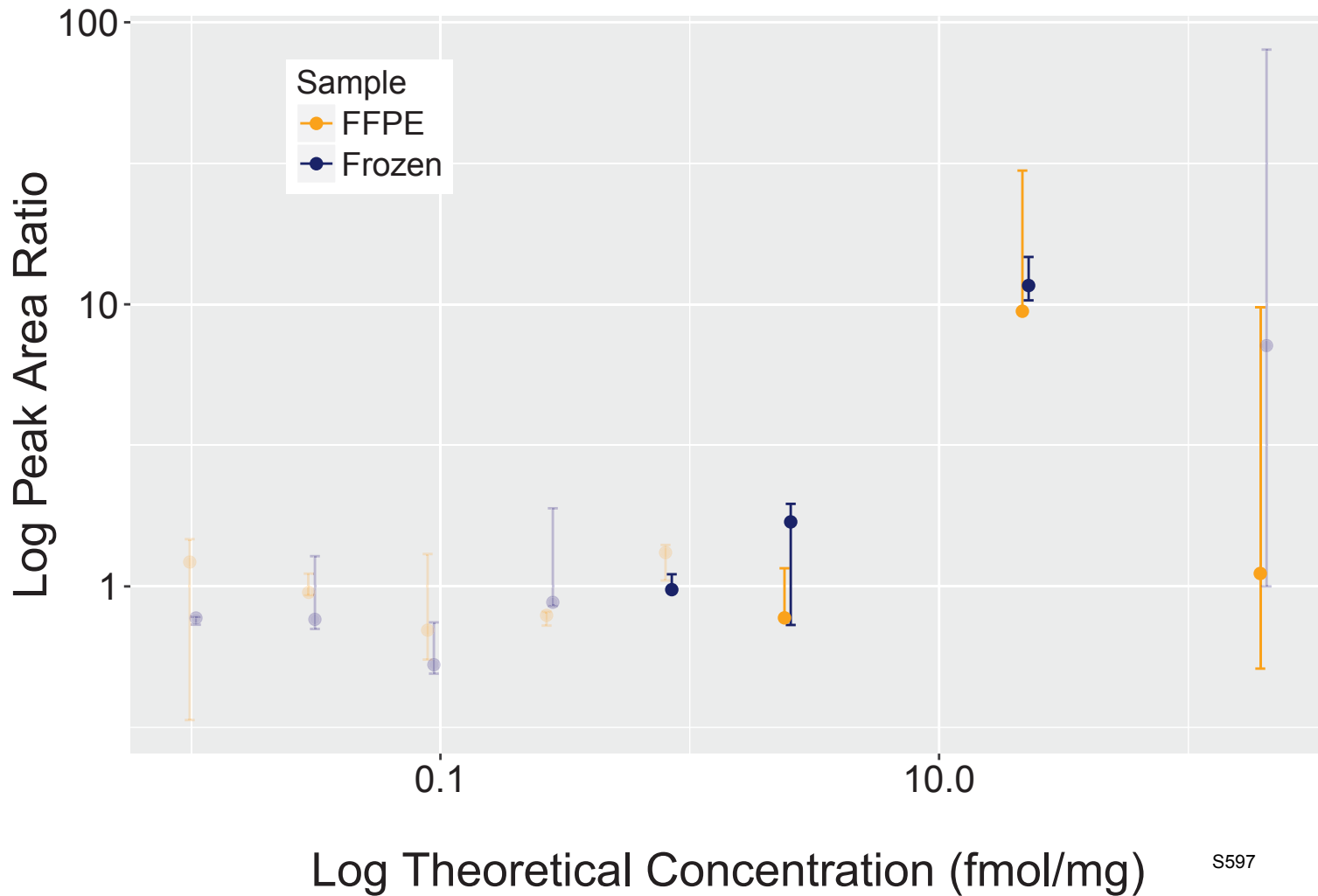
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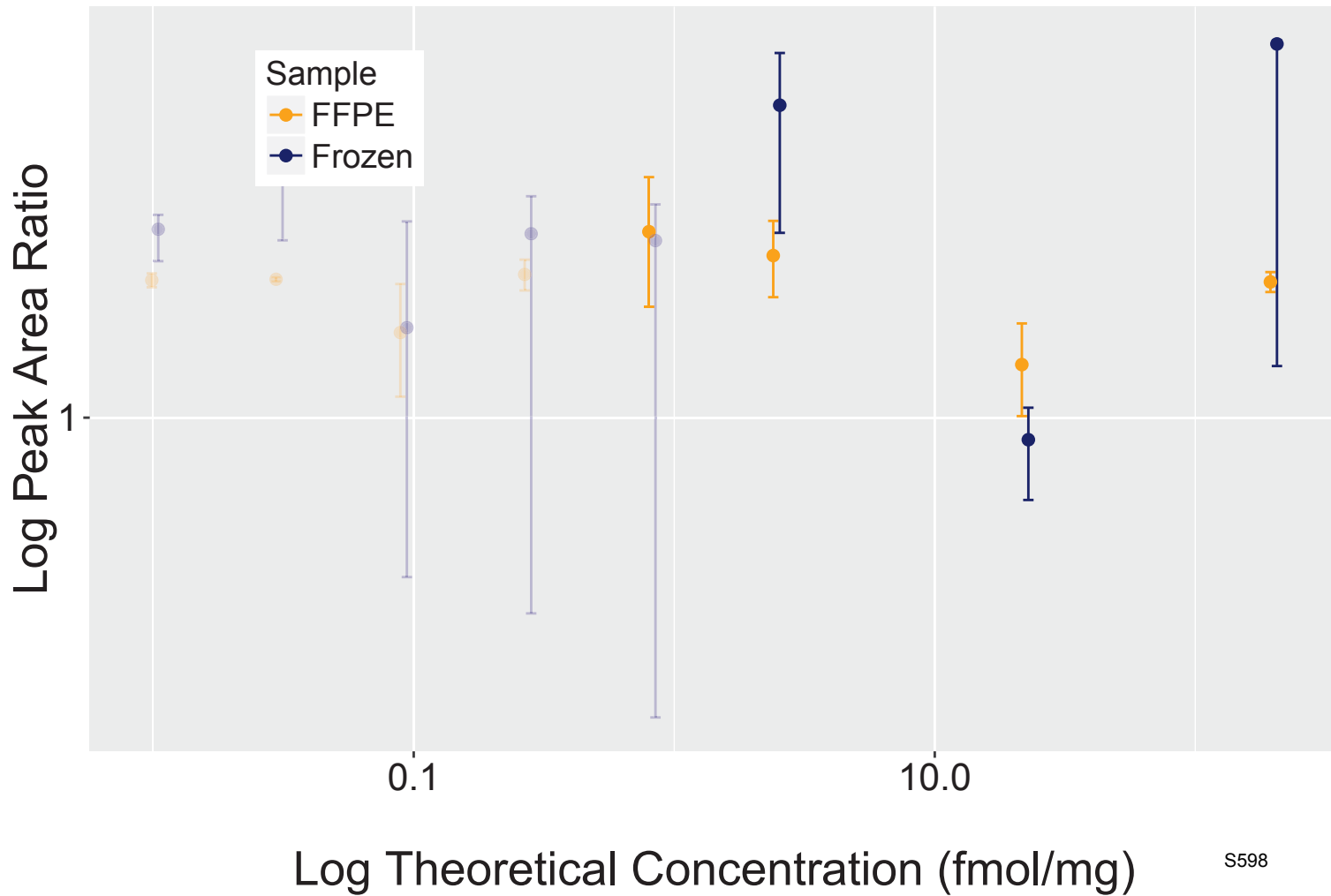
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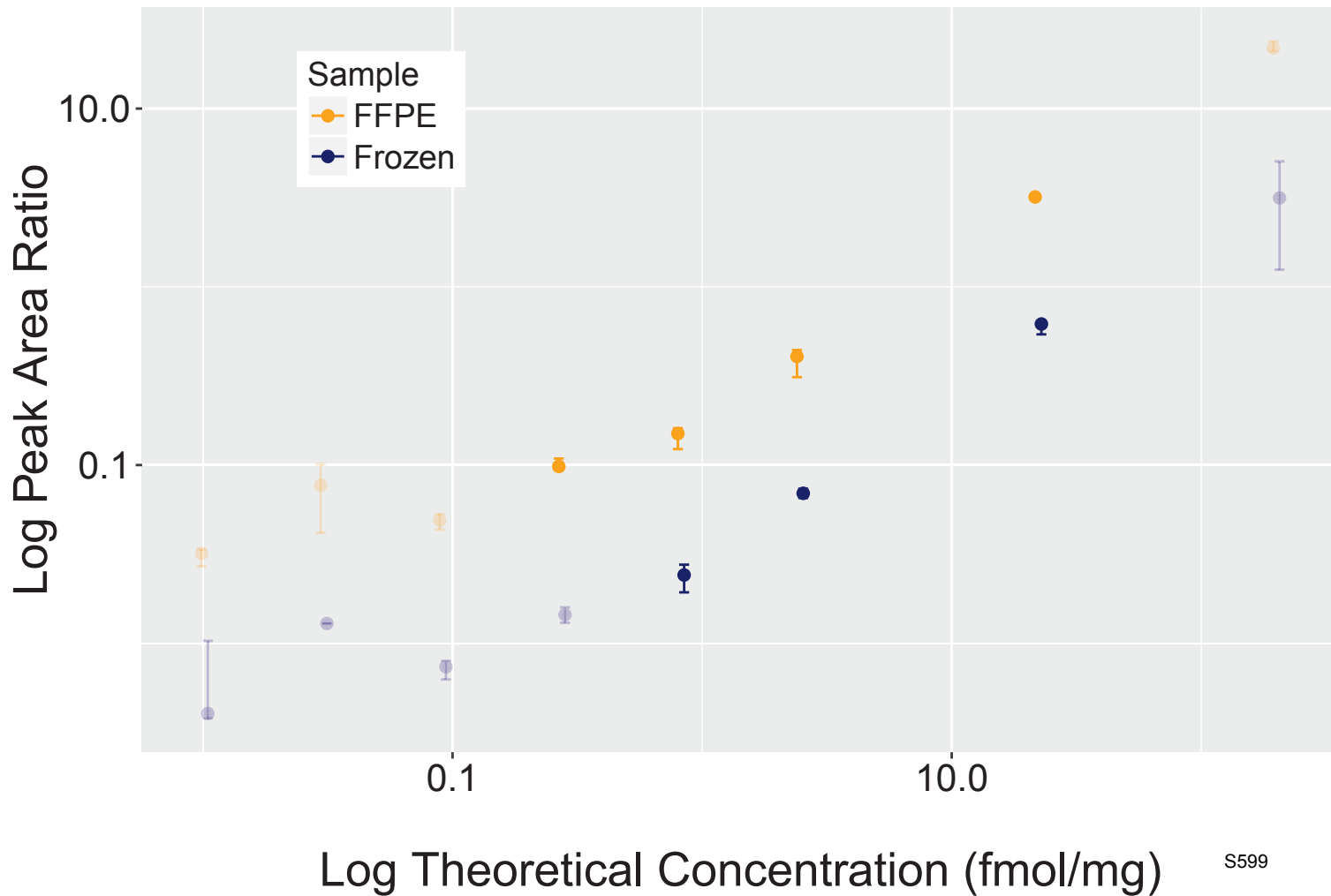
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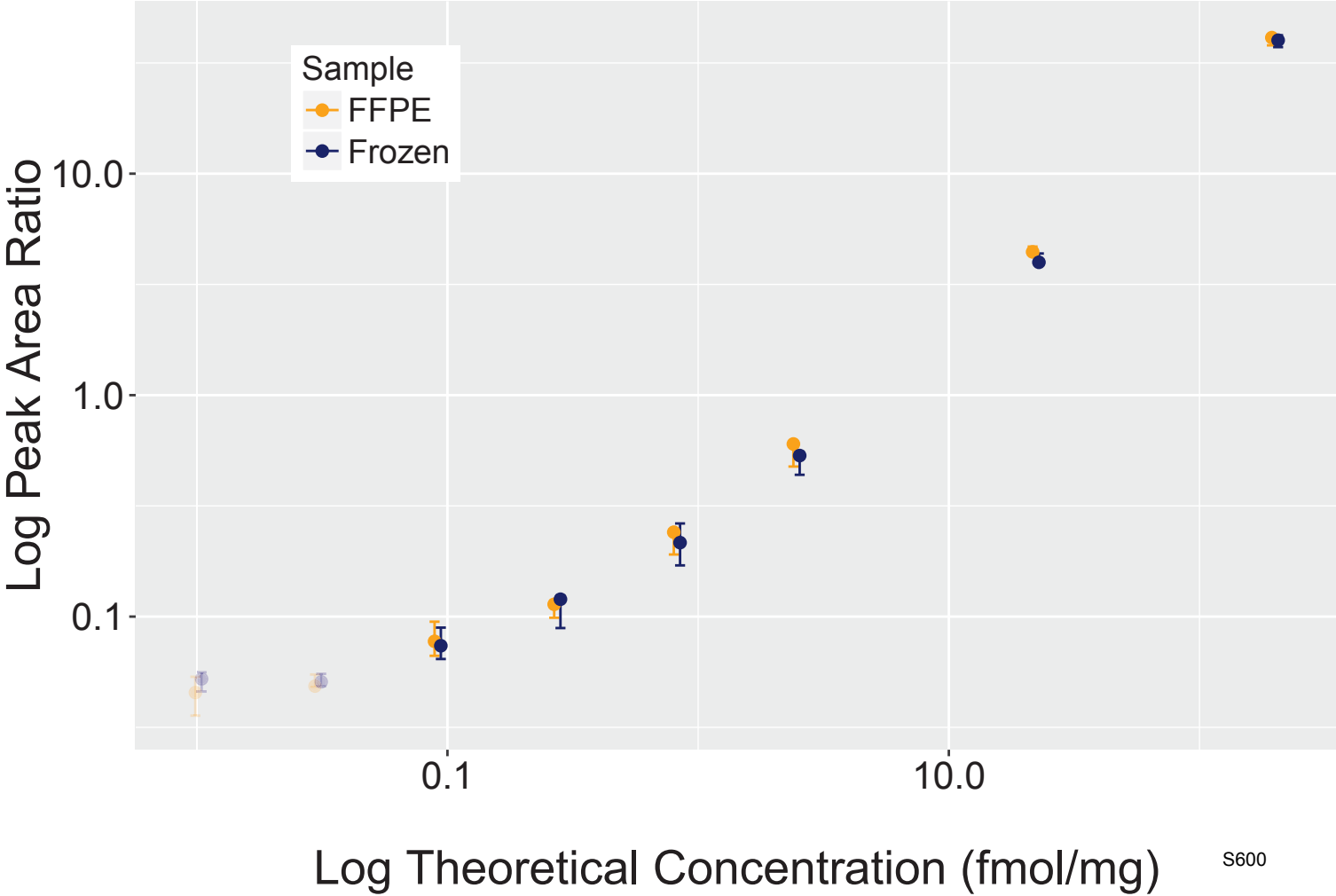
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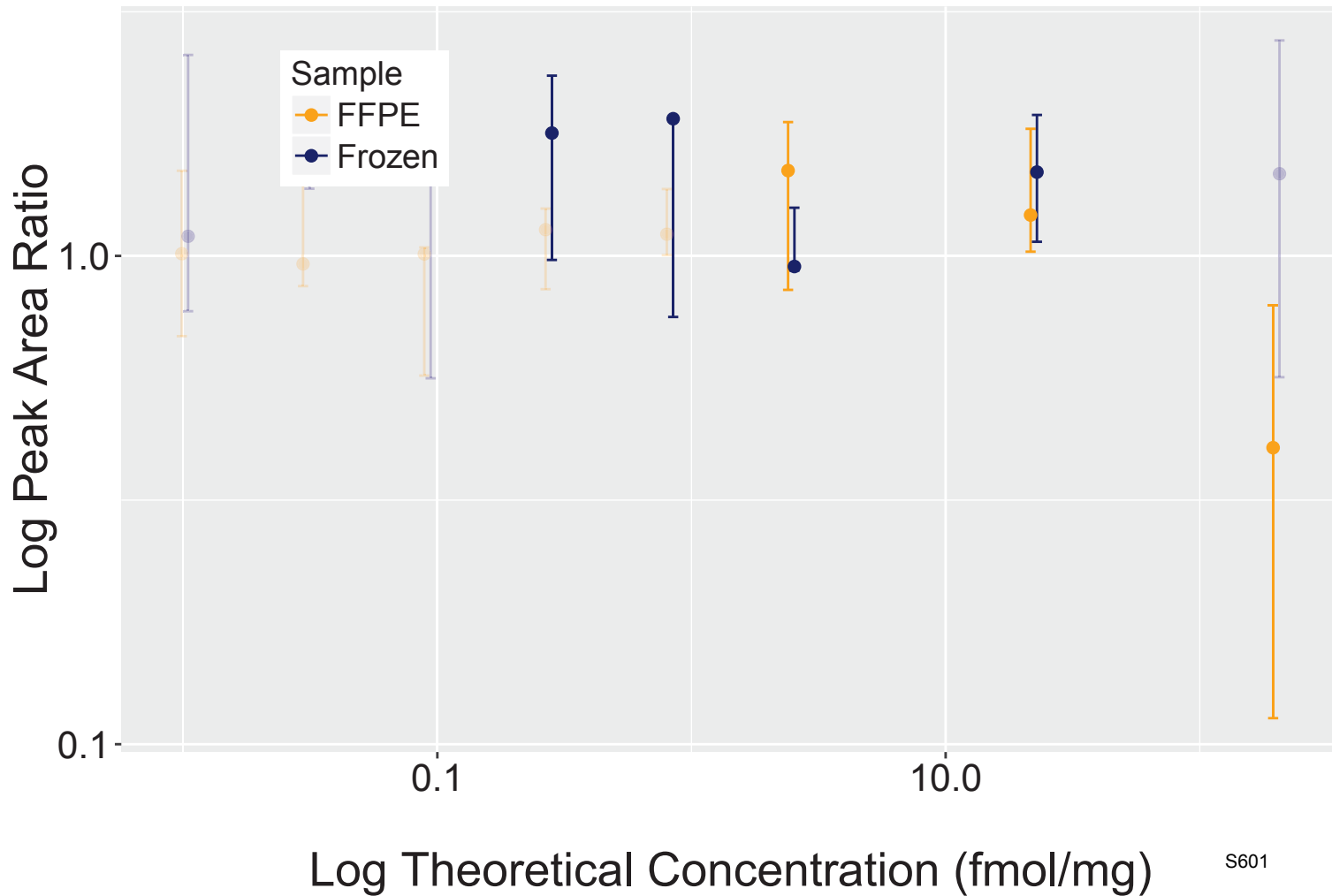
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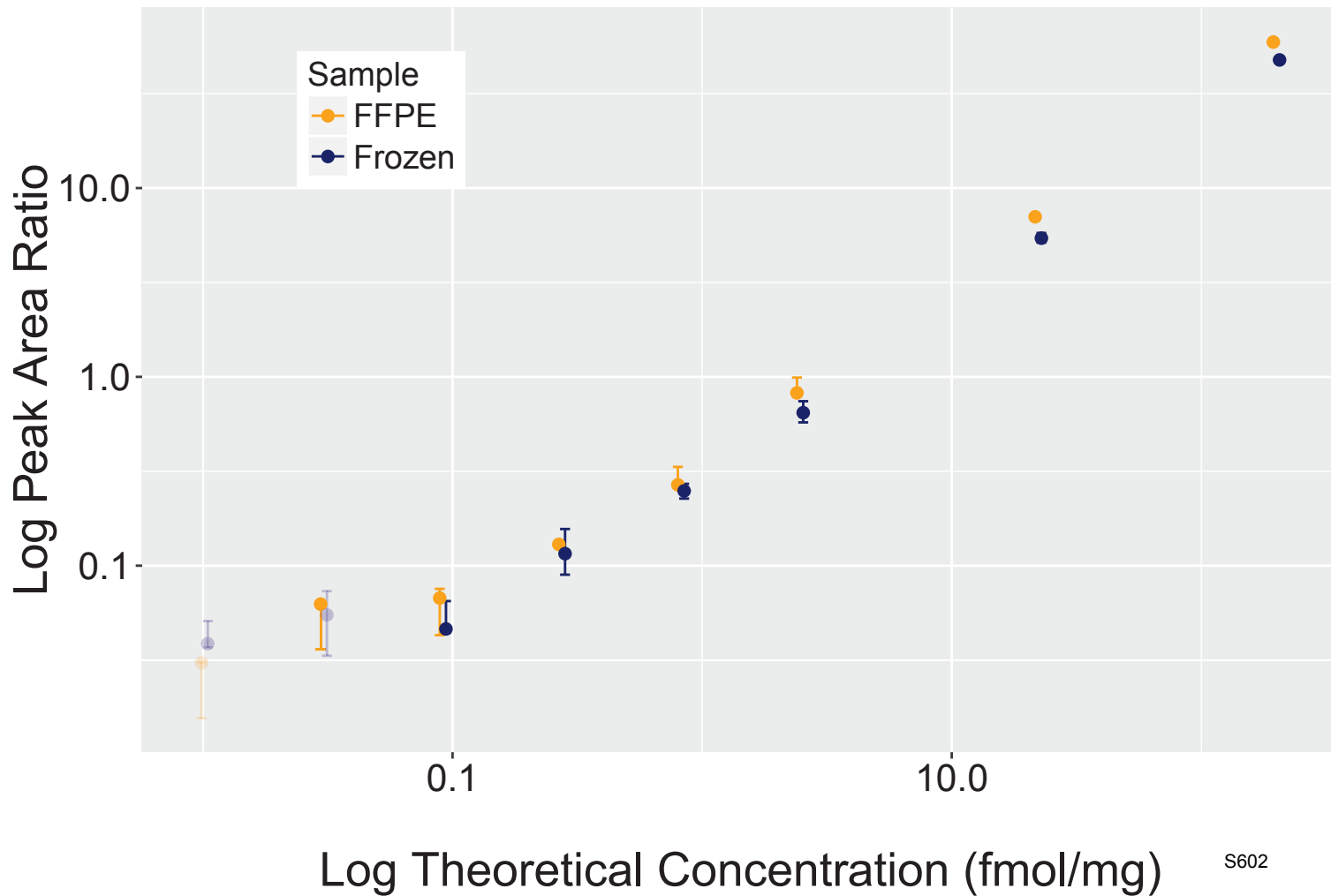
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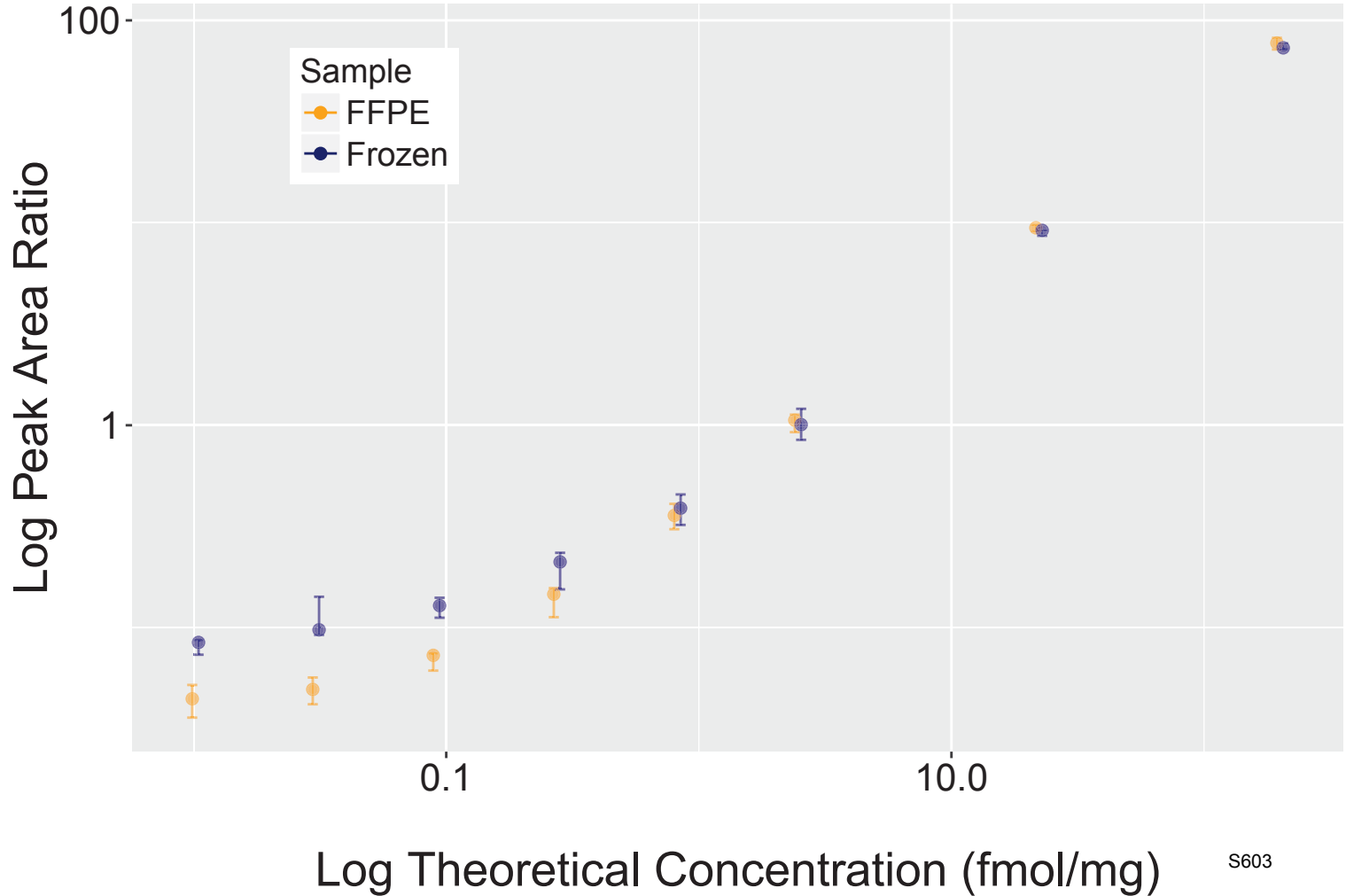
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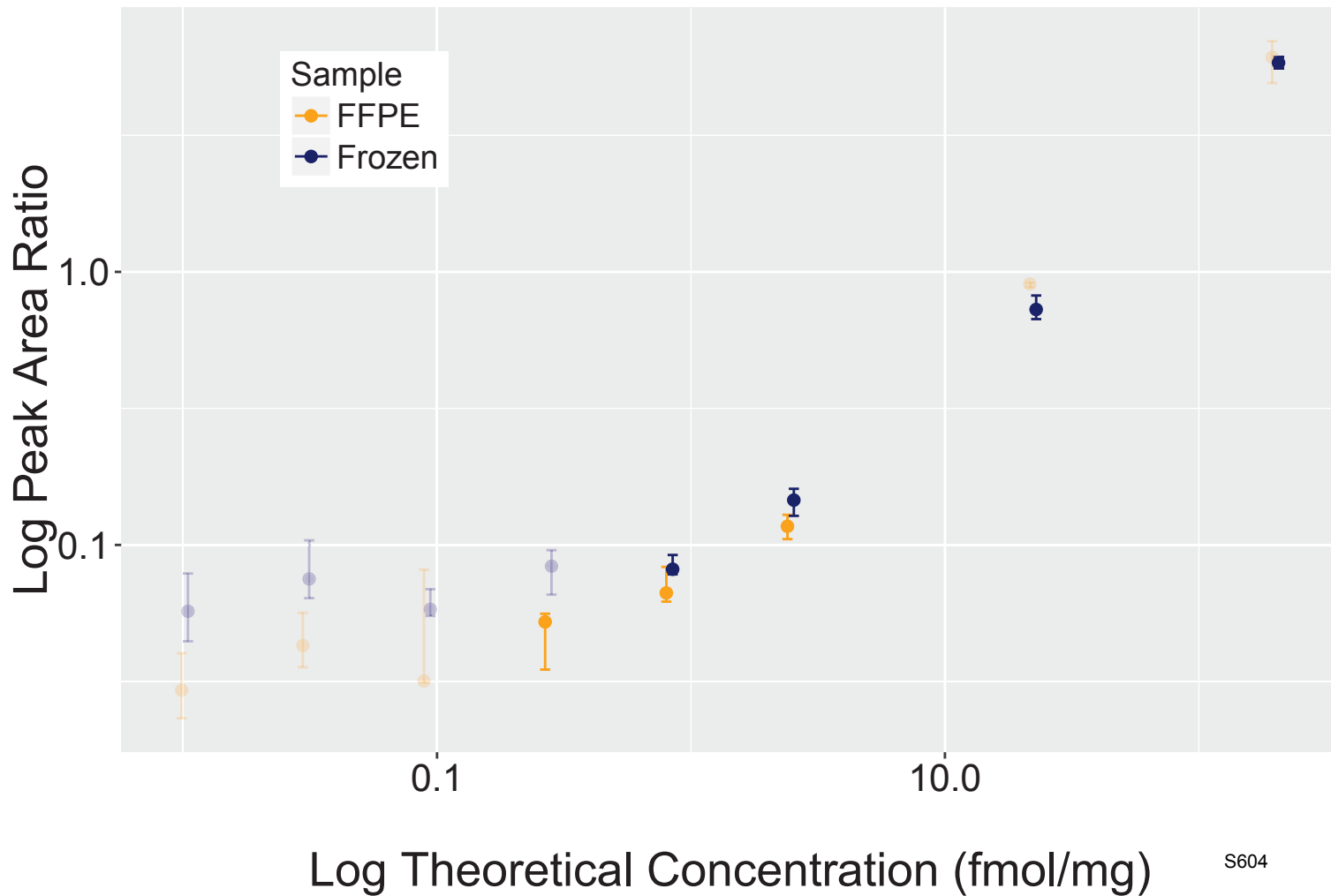
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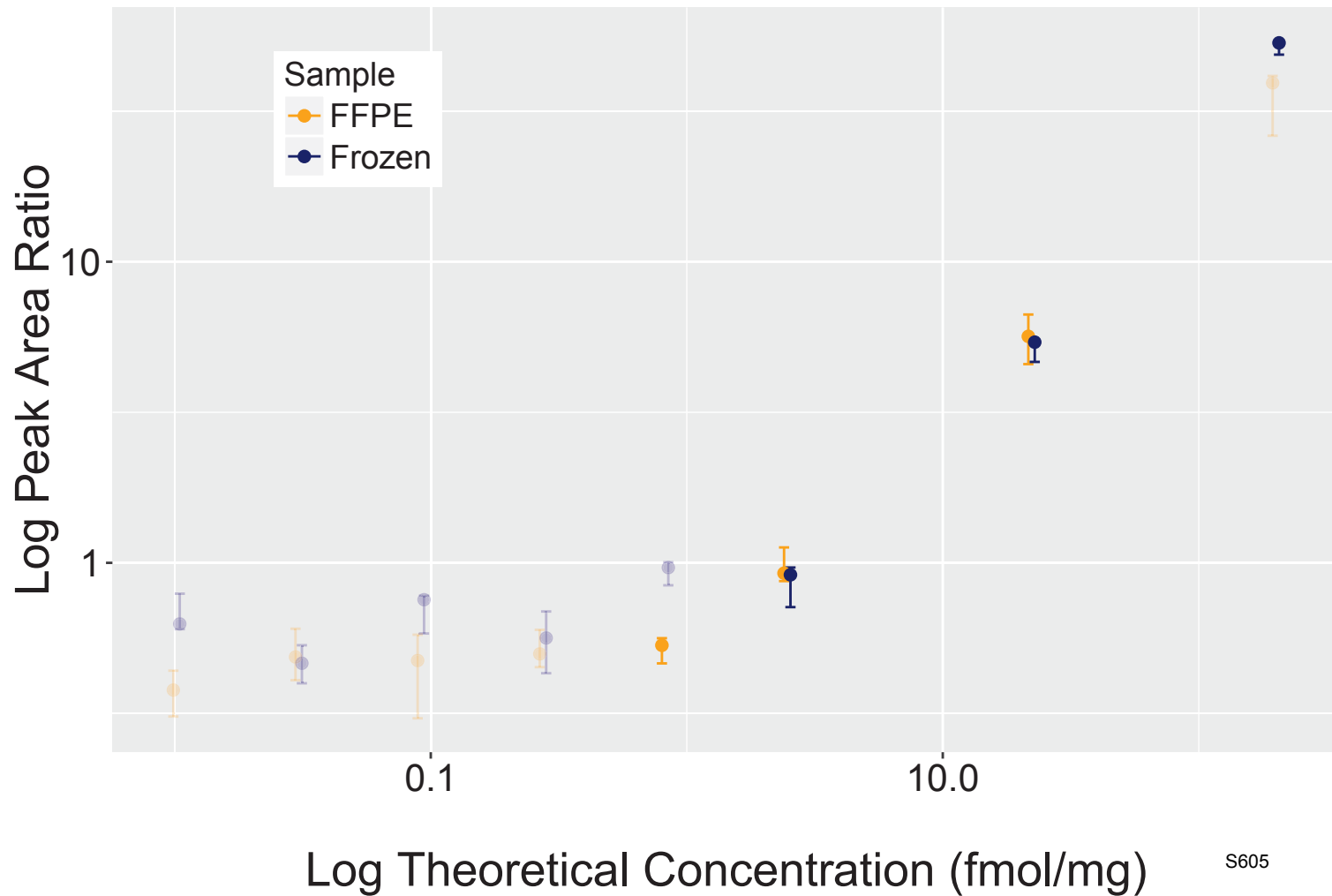
Analyte: GNL3.VGVIGFPNVGK



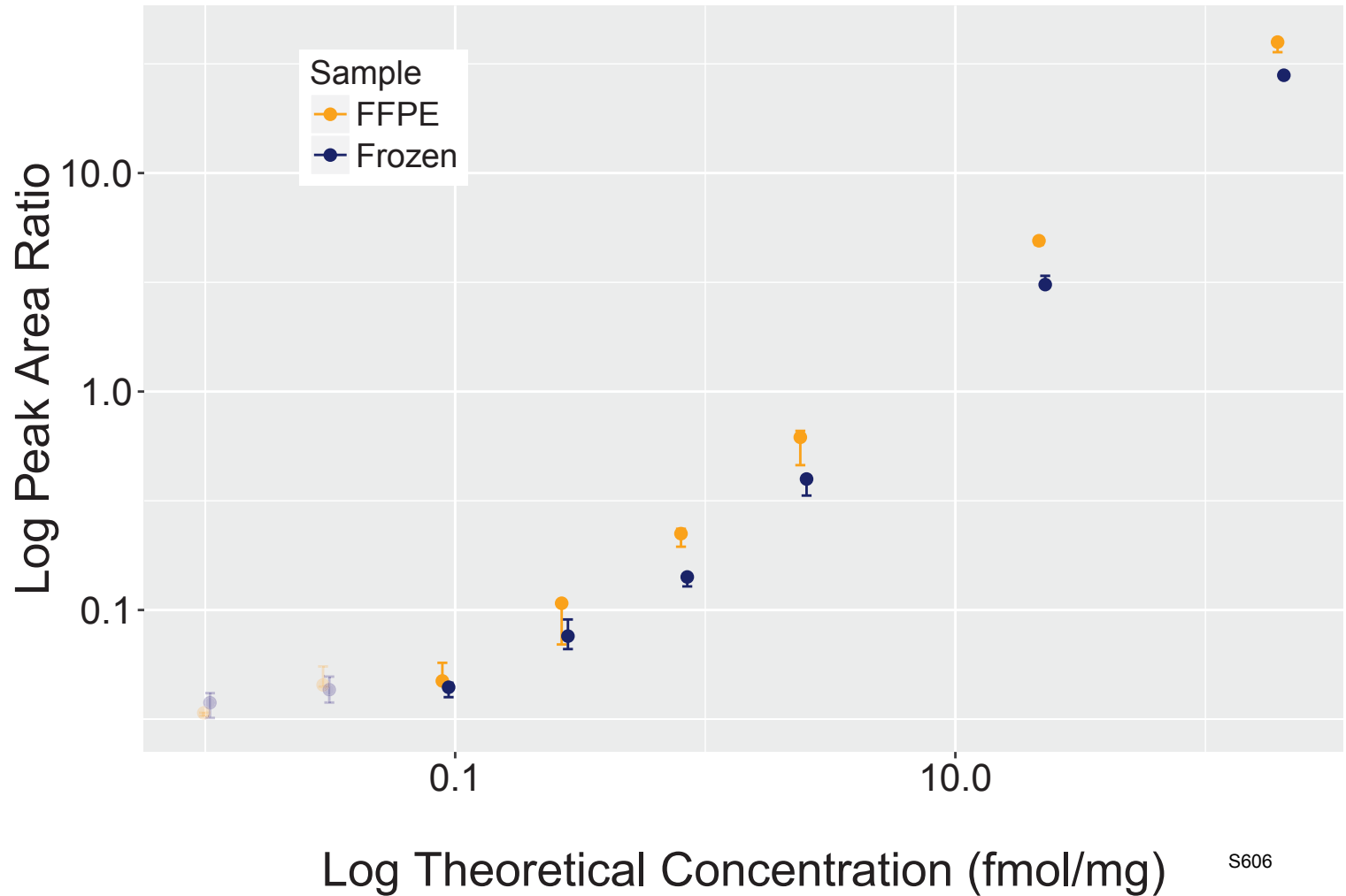
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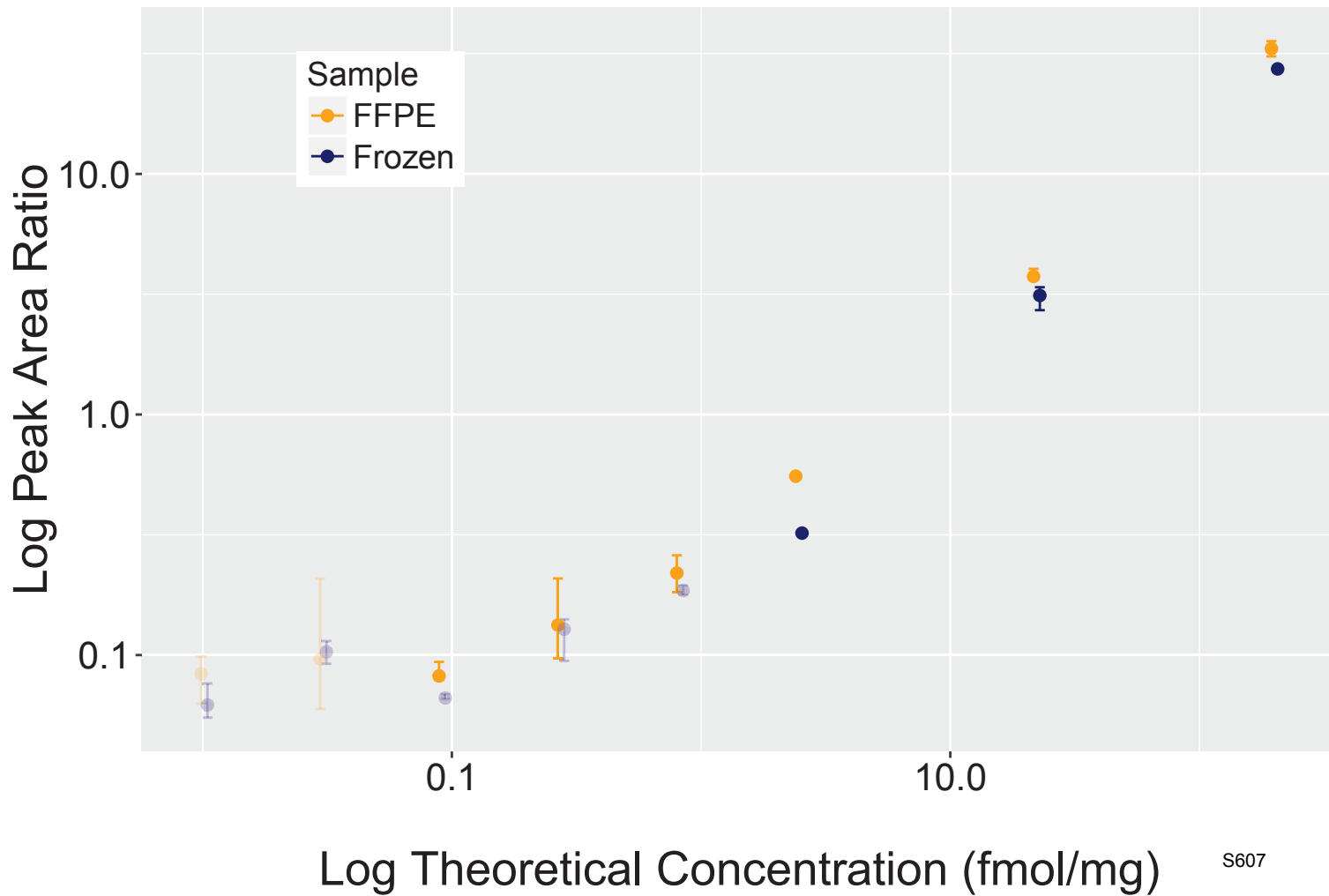
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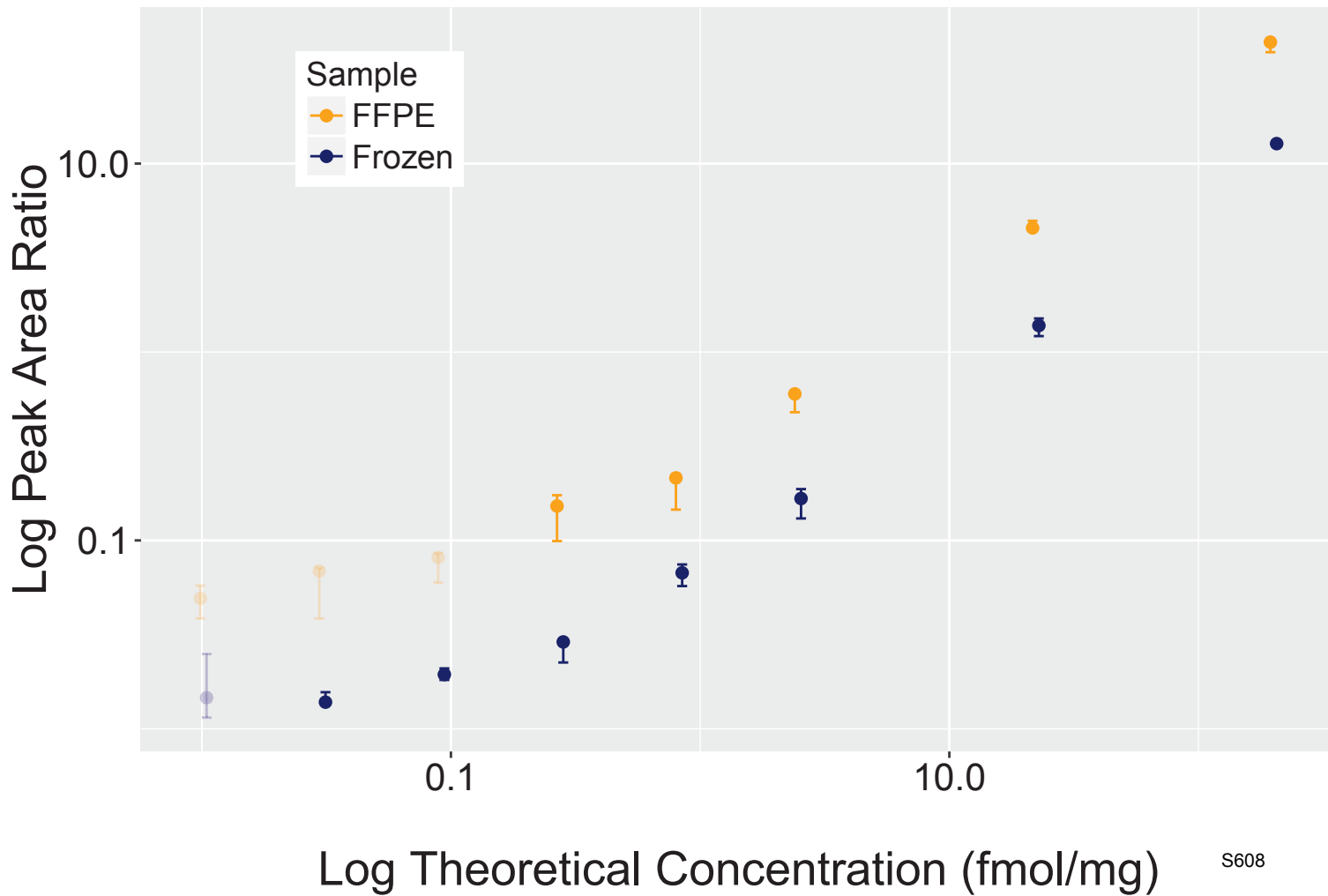
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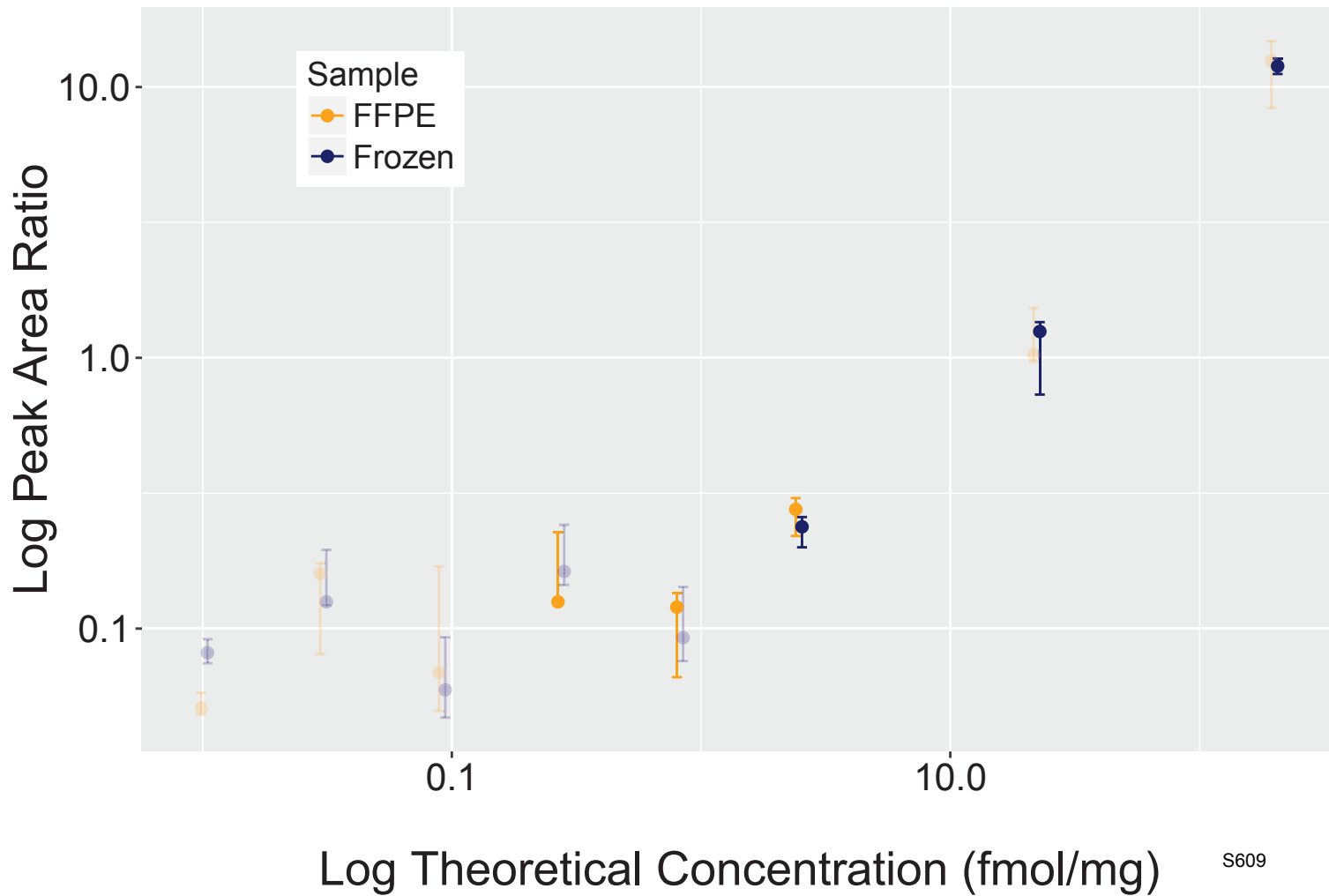
Analyte: ISYNA1.VIVLWTANTER



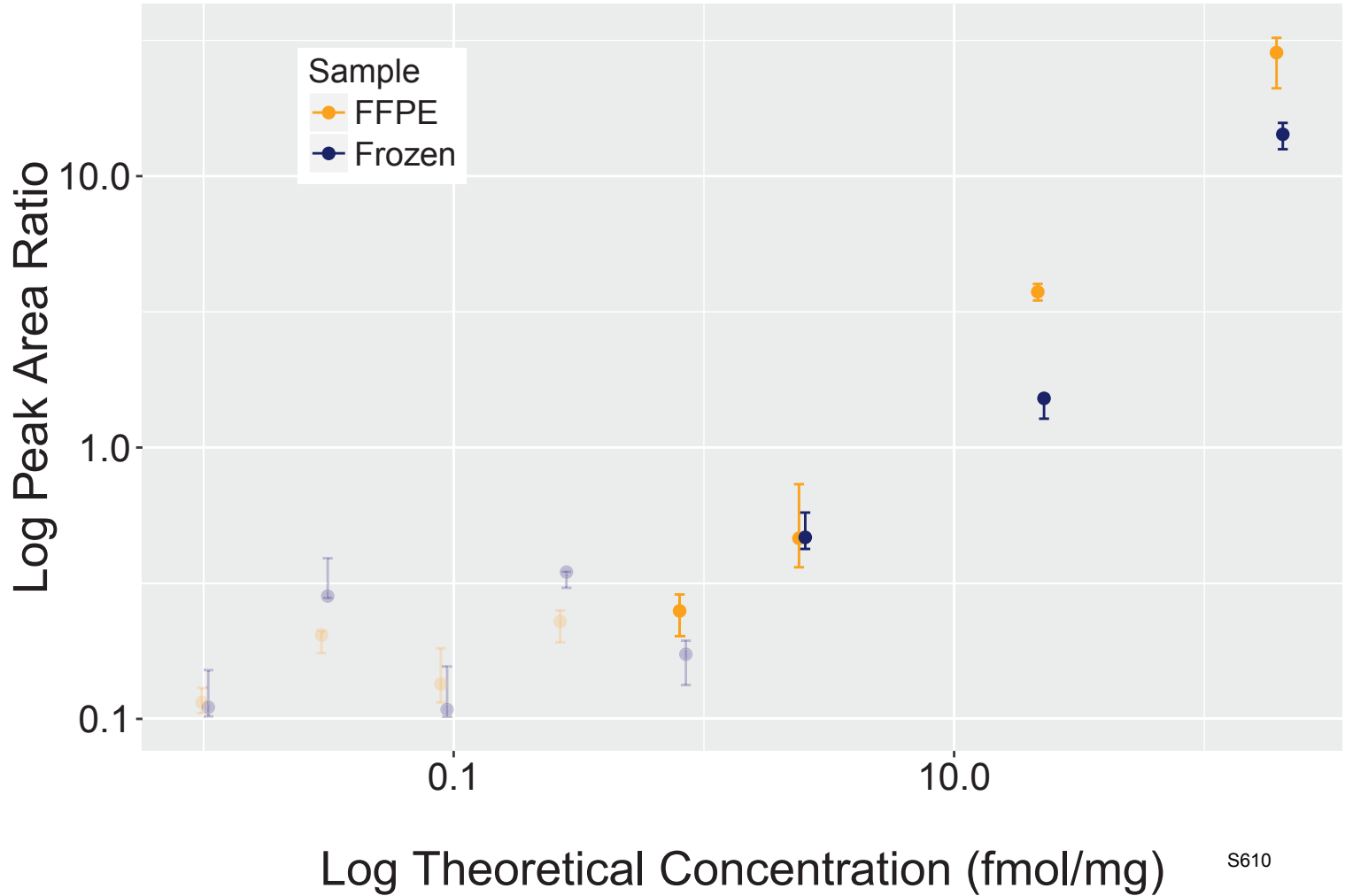
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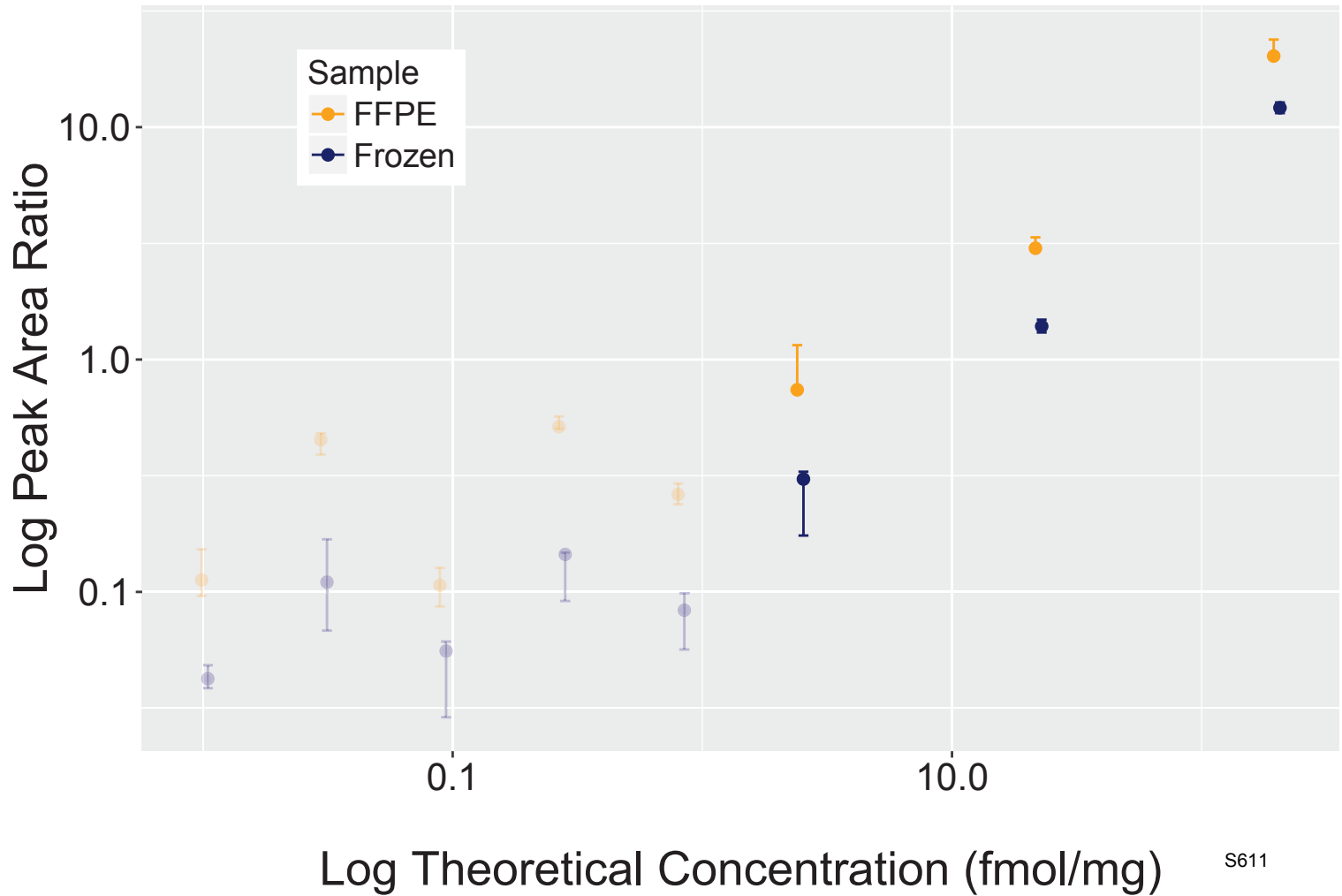
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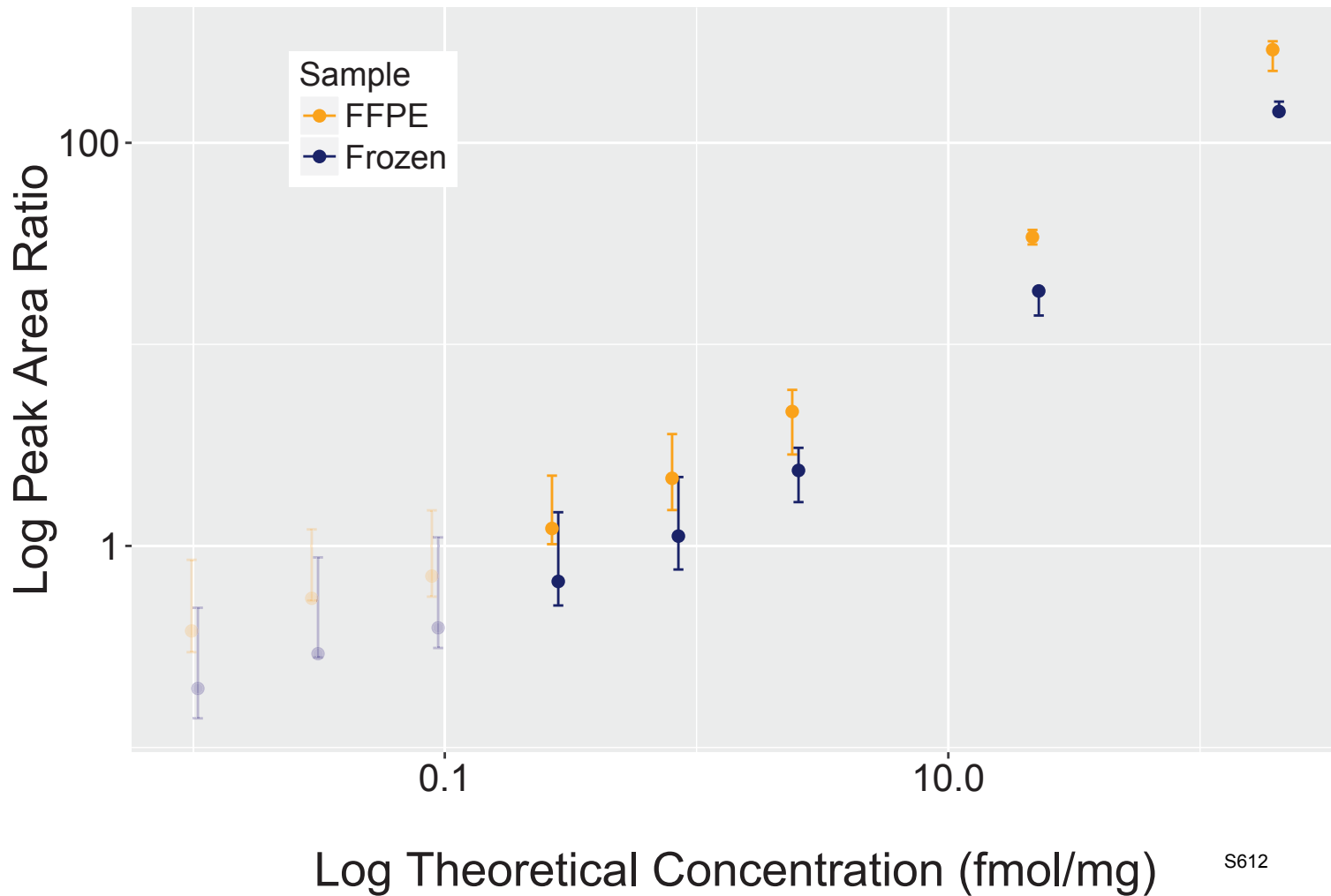
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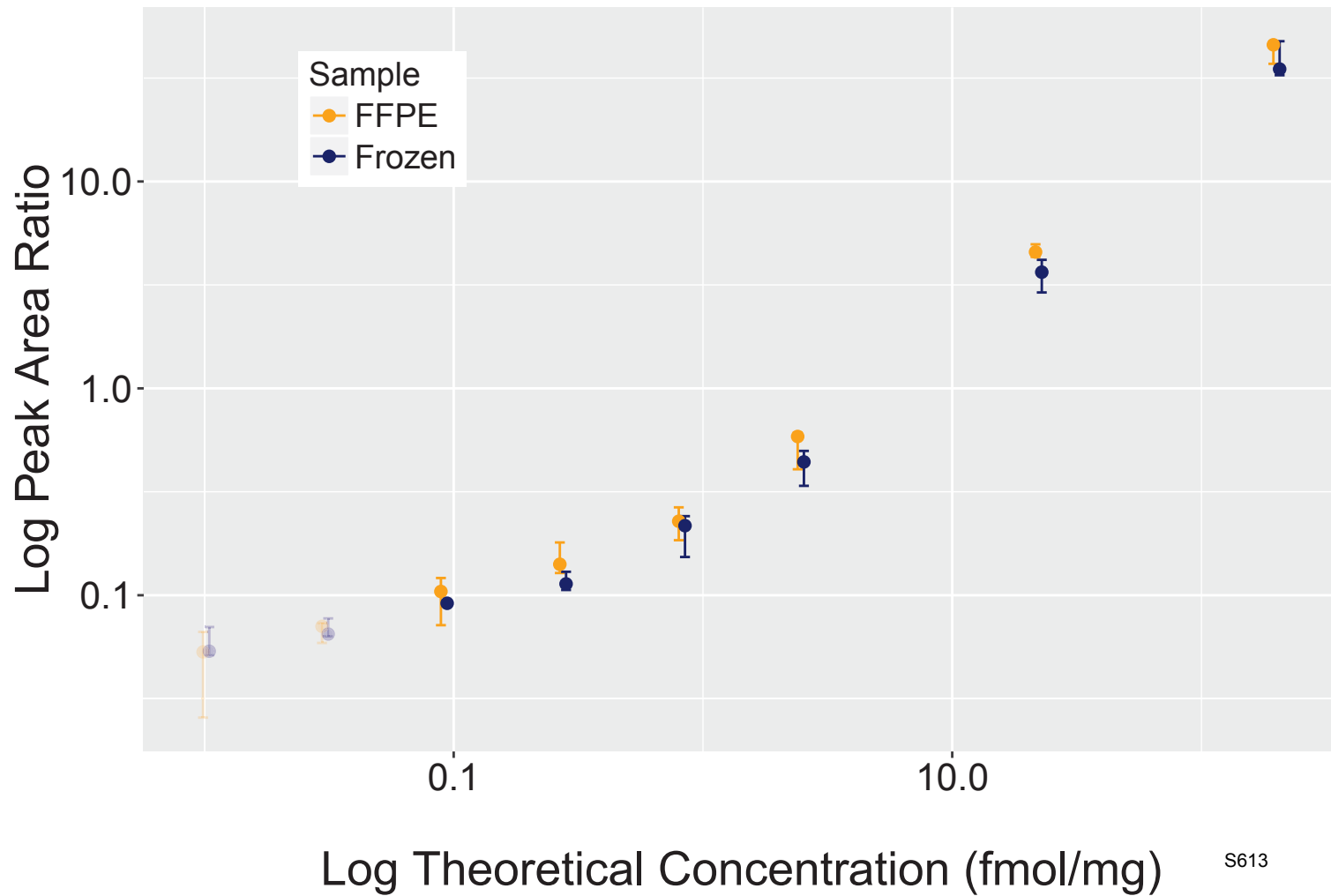
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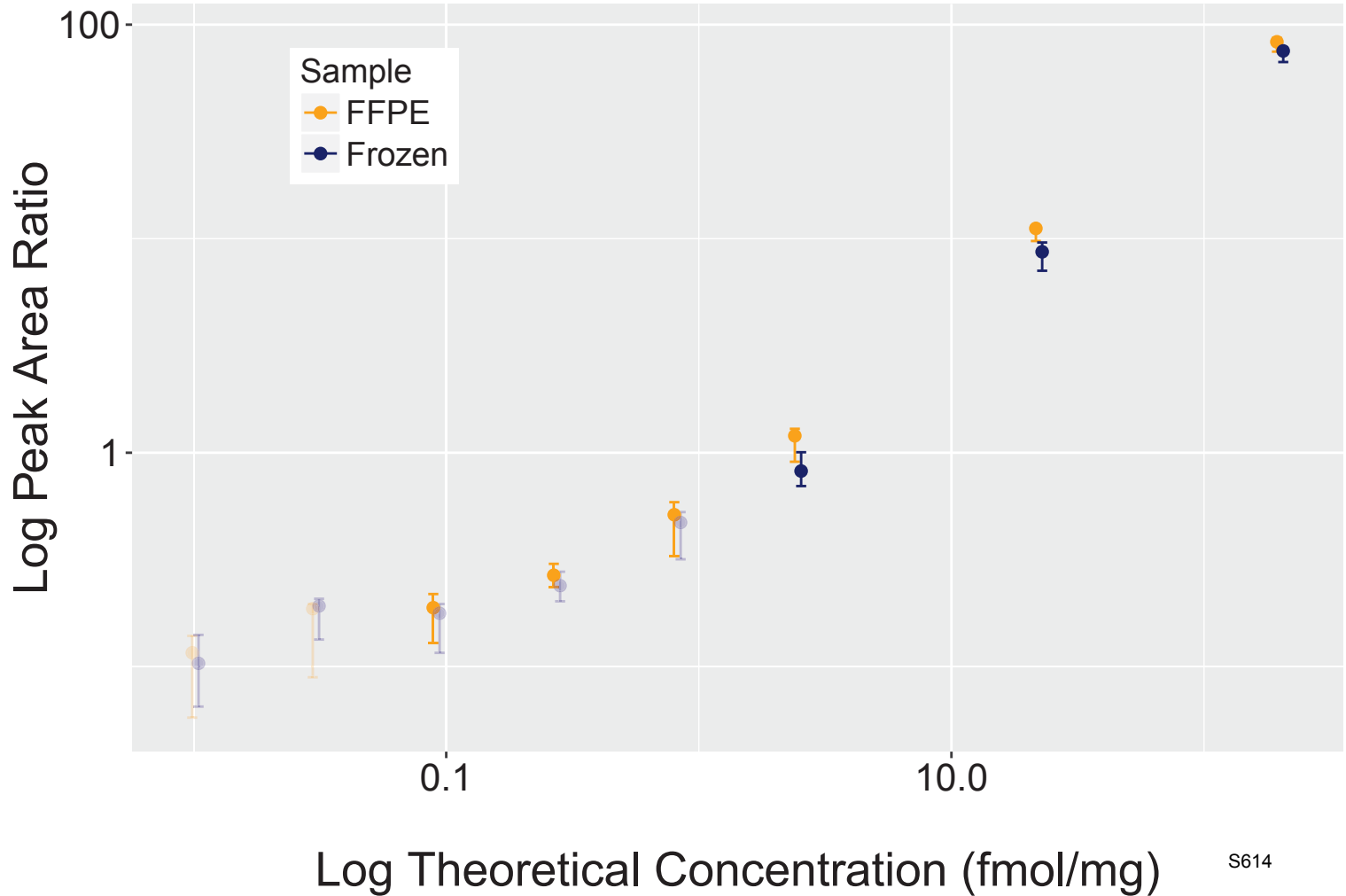
Analyte: HEXB.VLDIIATINK



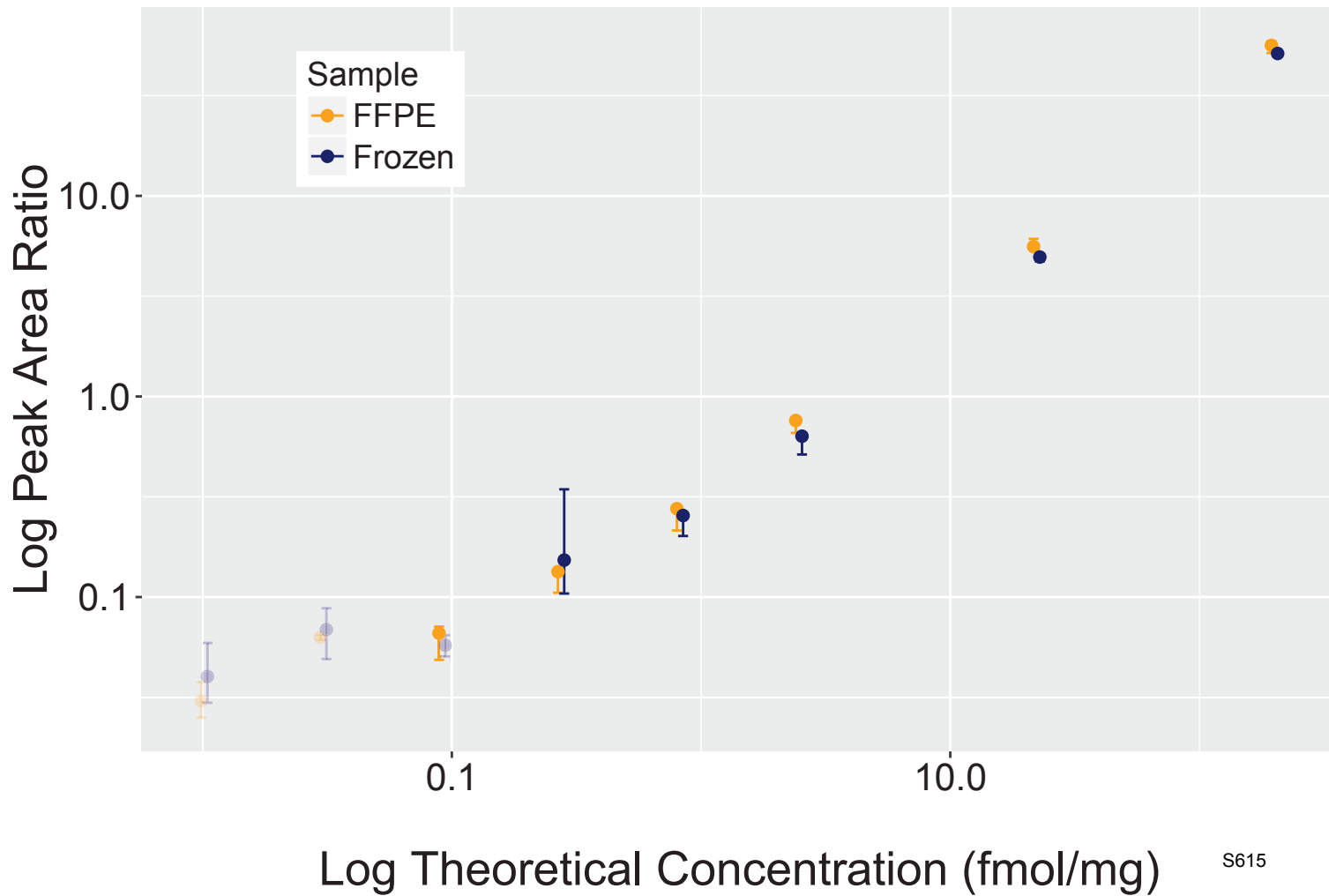
Analyte: SSH3.VLDVSDLESVTSK



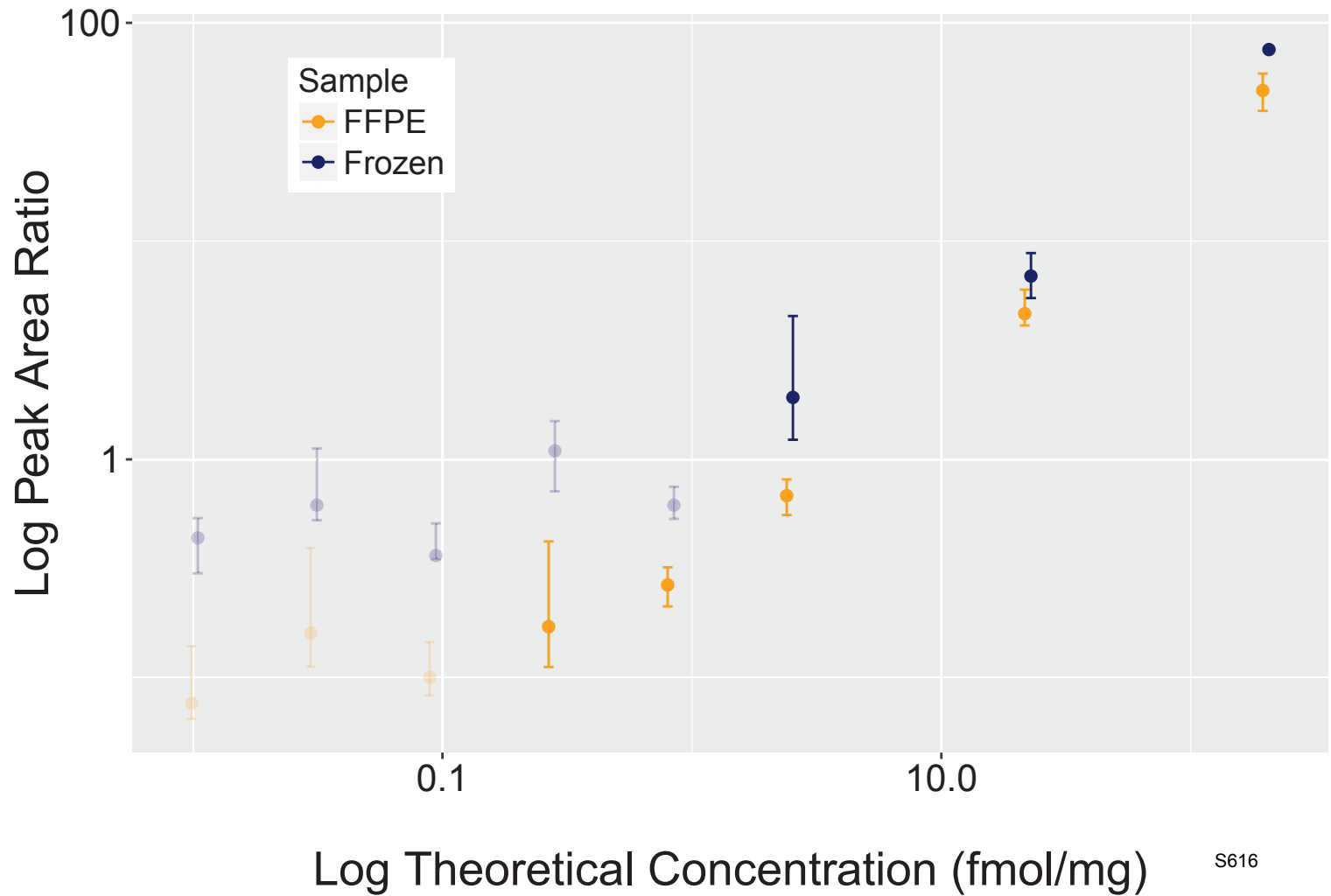
Analyte: AIP.VLELDPALAPVVS



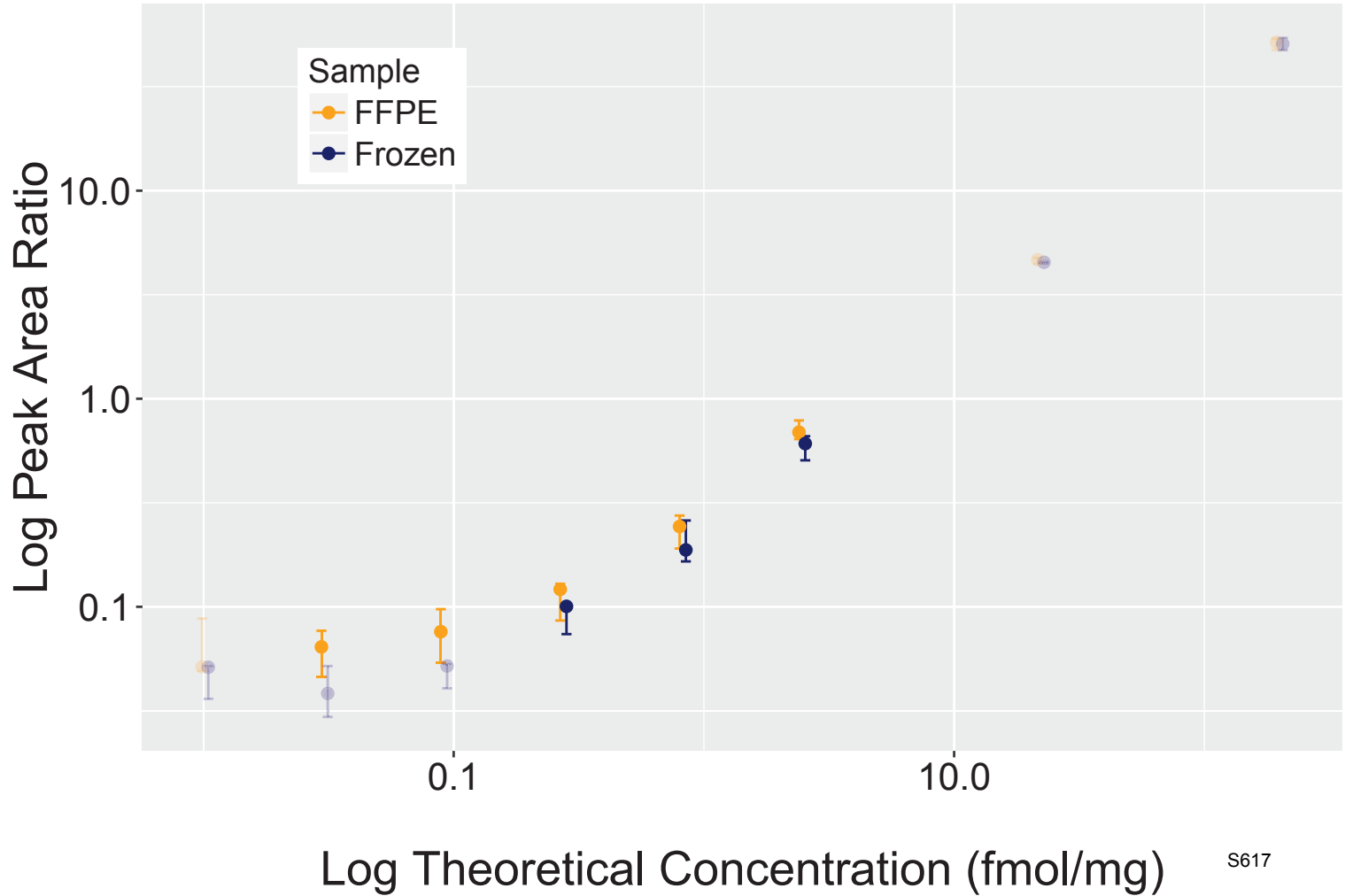
Analyte: ACOT7.VLEVPPVVYSR



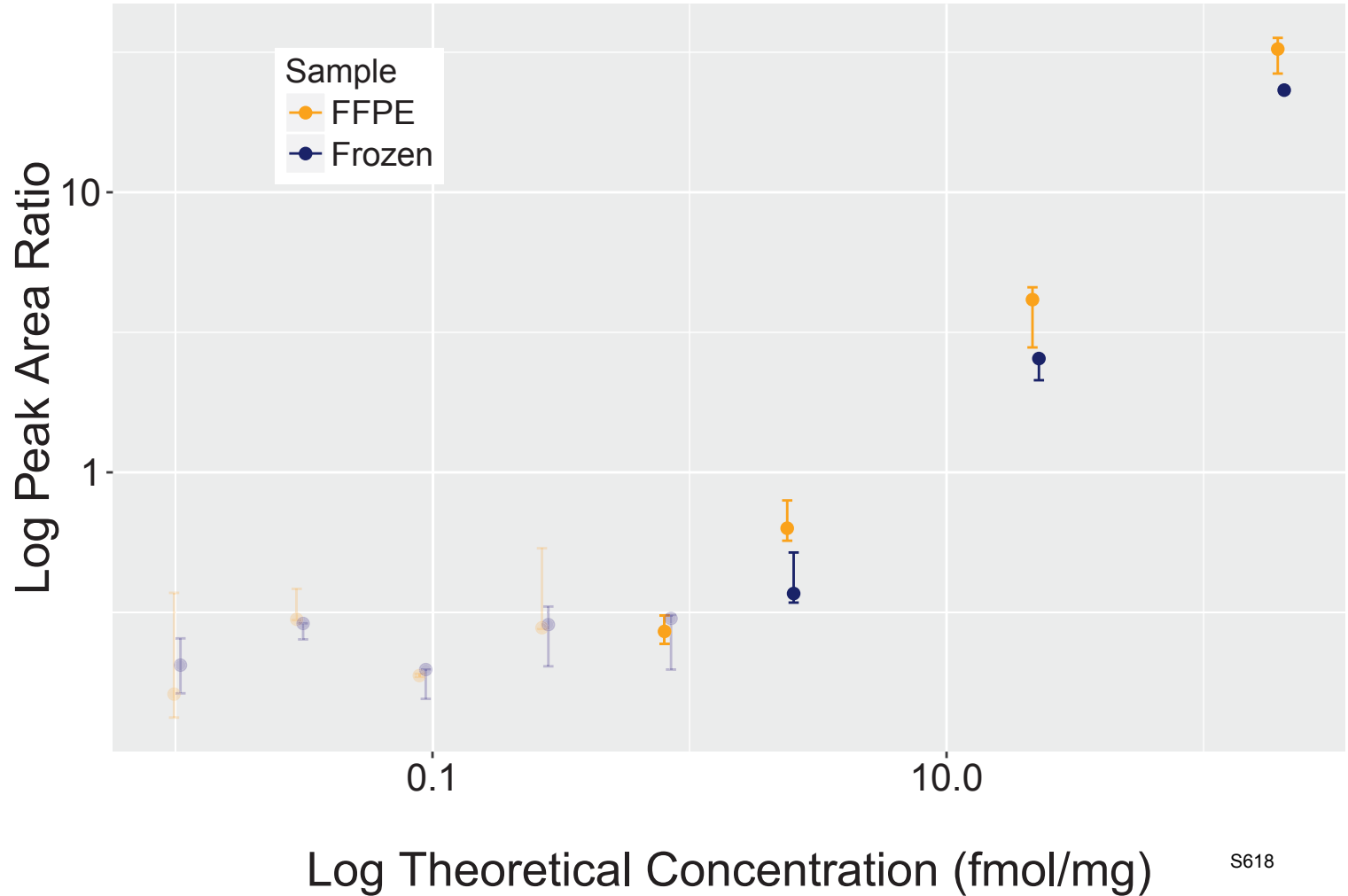
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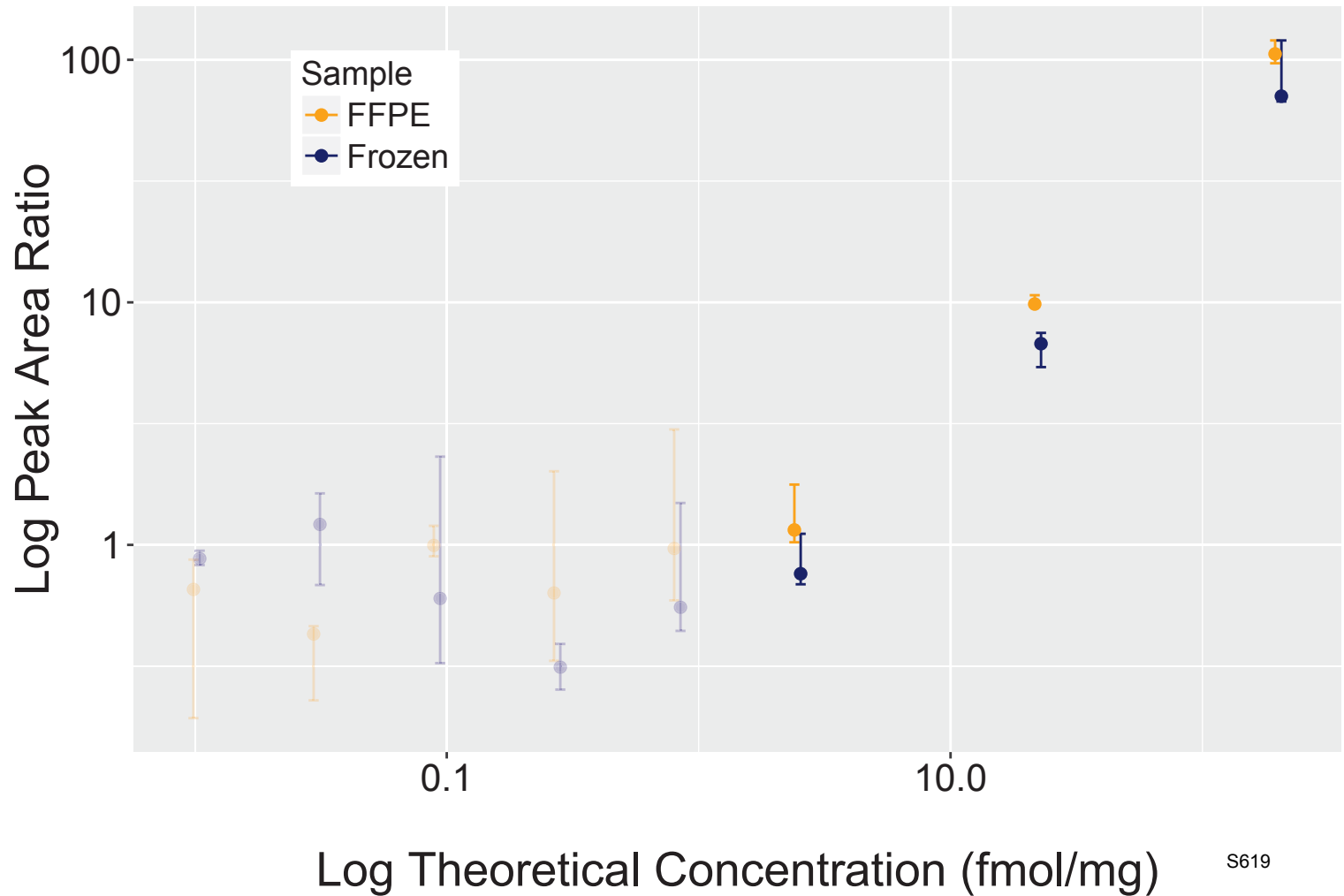
Analyte: CAD.VLGTSPEAIDSAENR



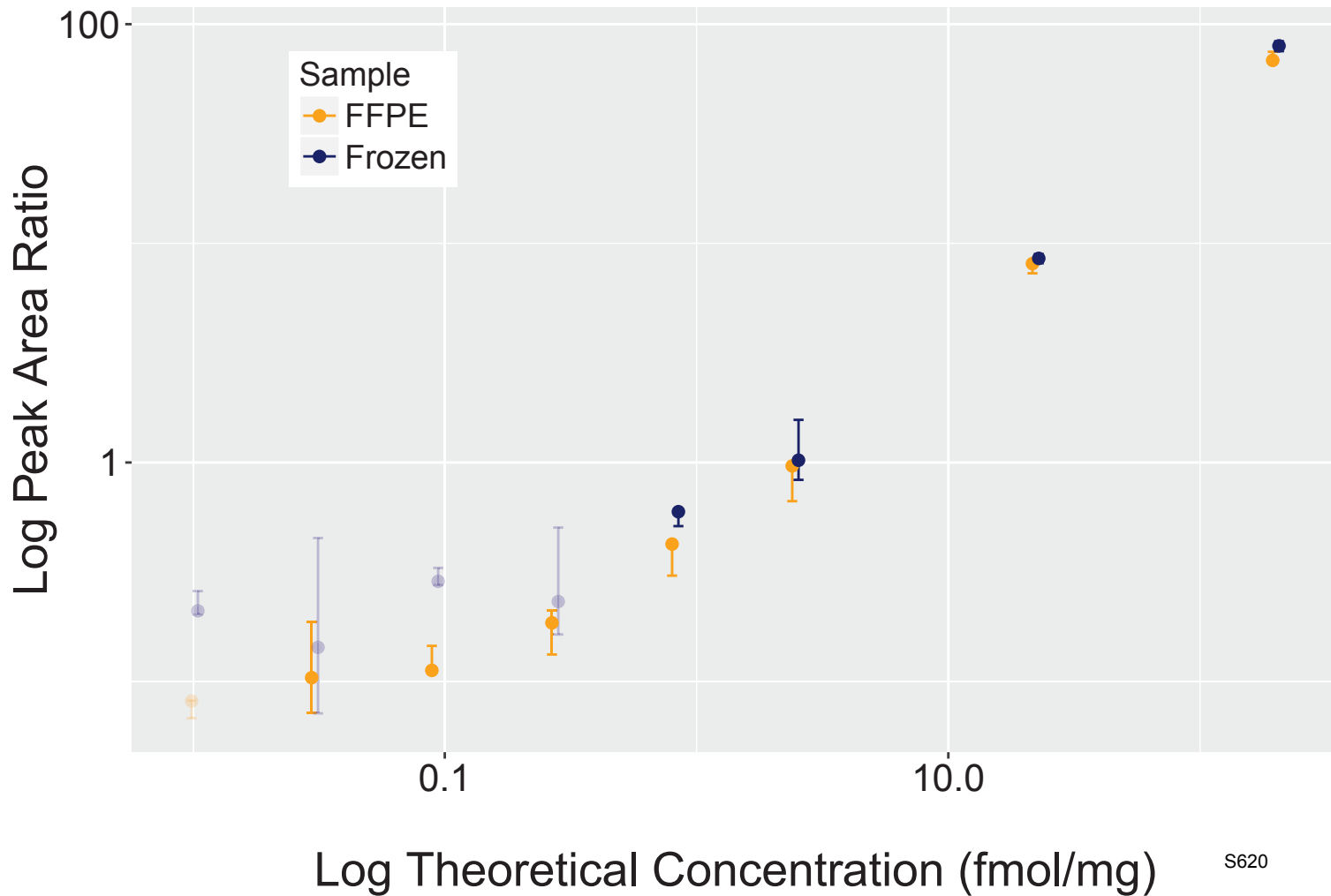
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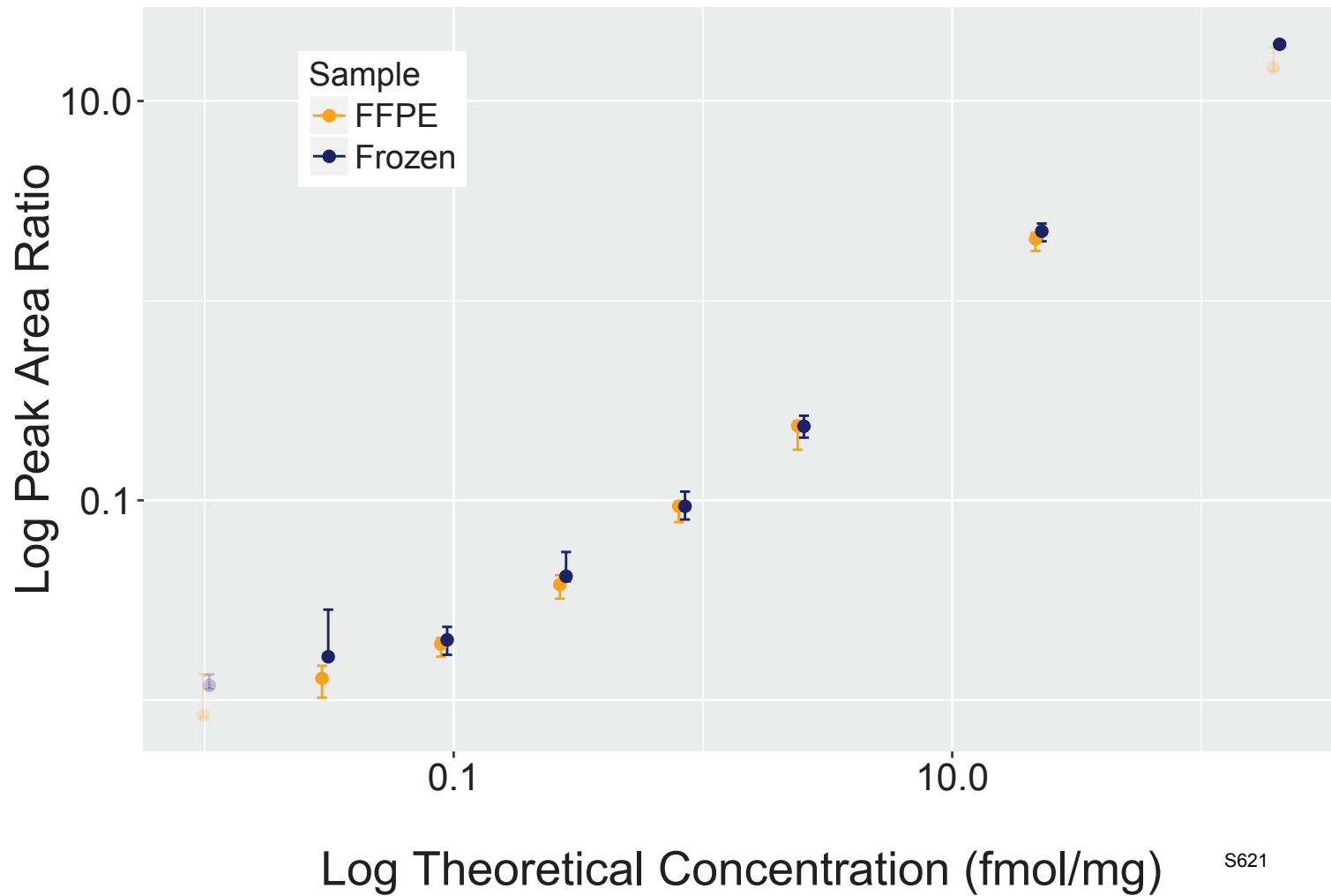
Analyte: NT5E.VLPVGDEVVGVGYTSK



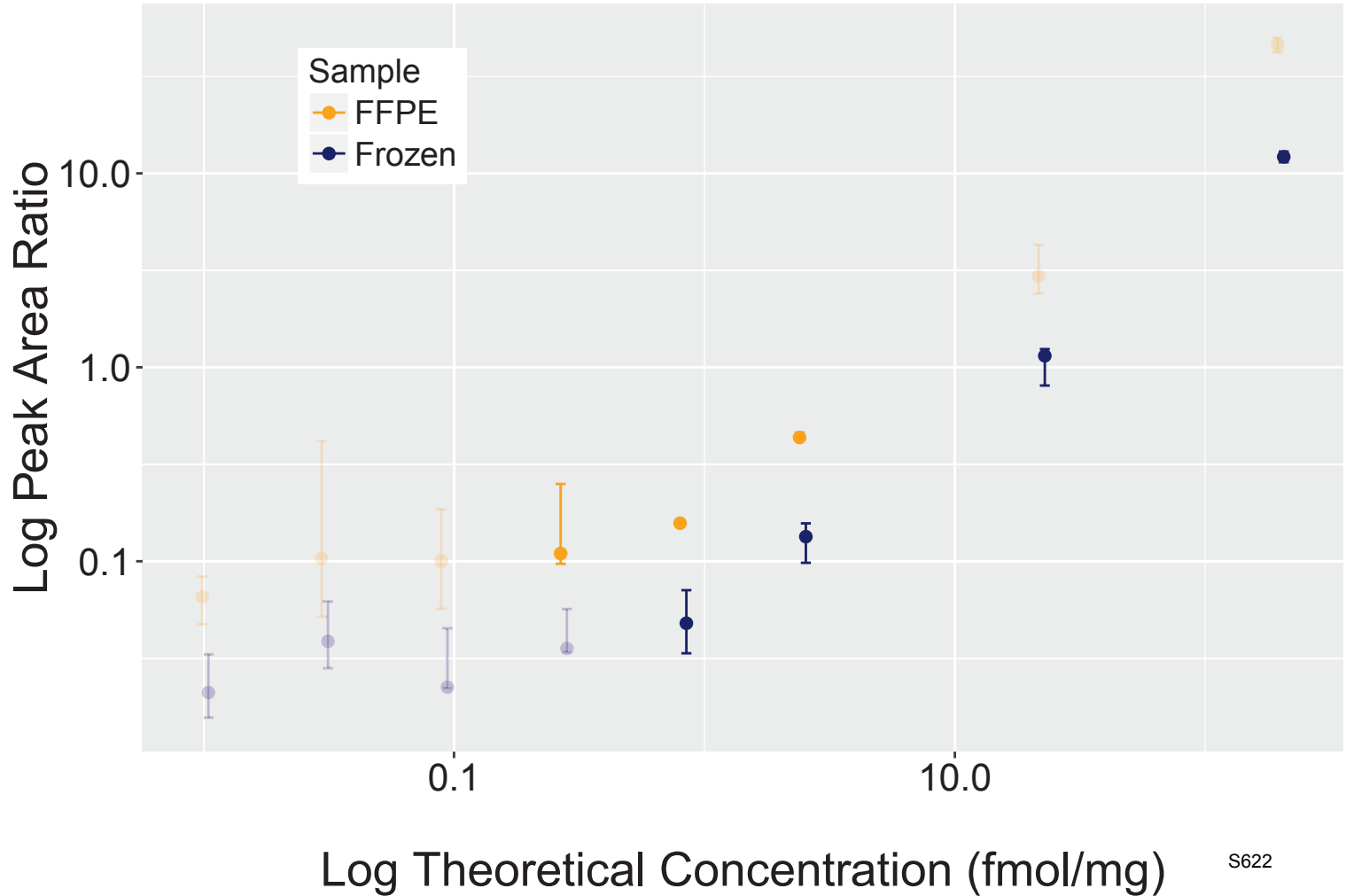
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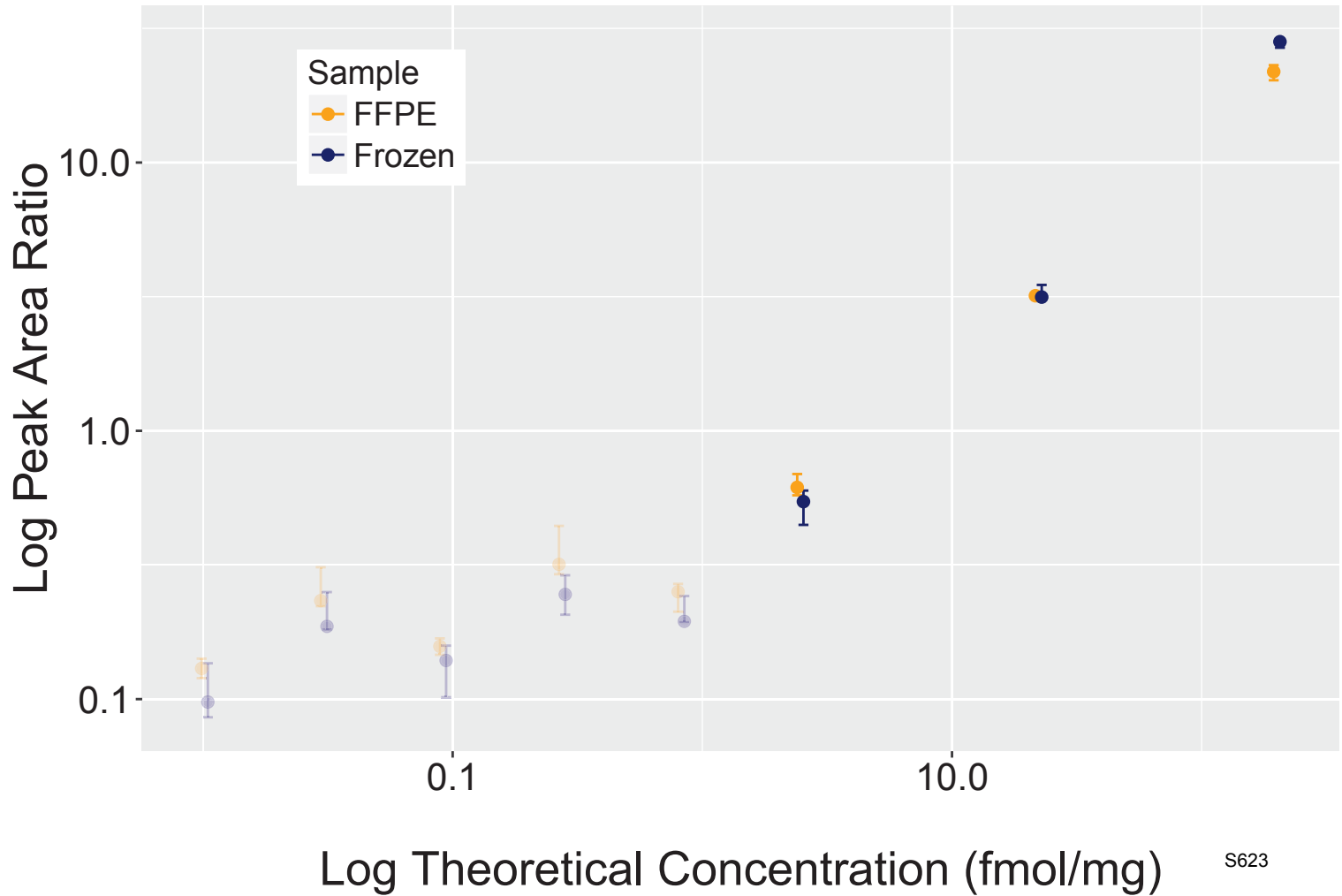
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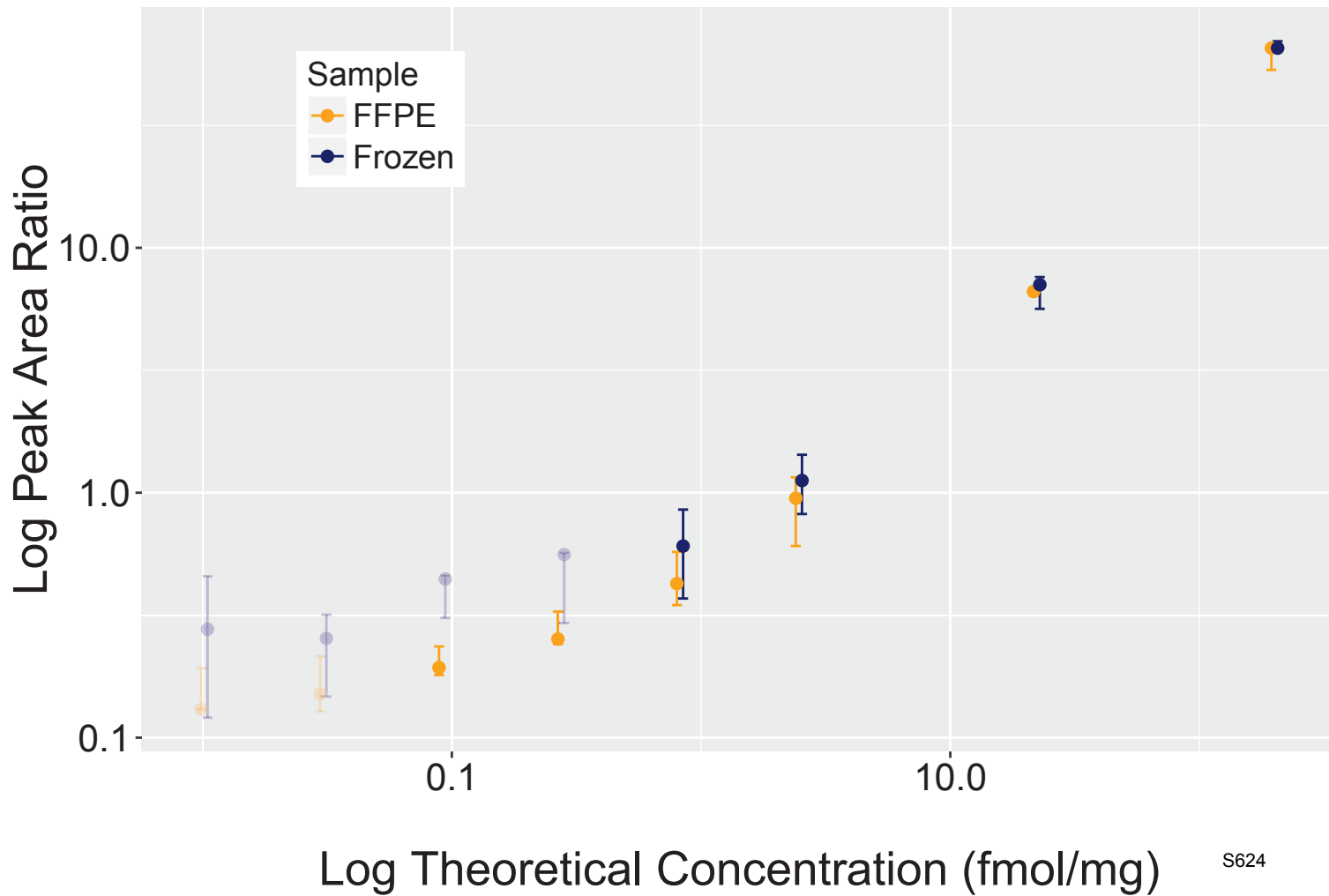
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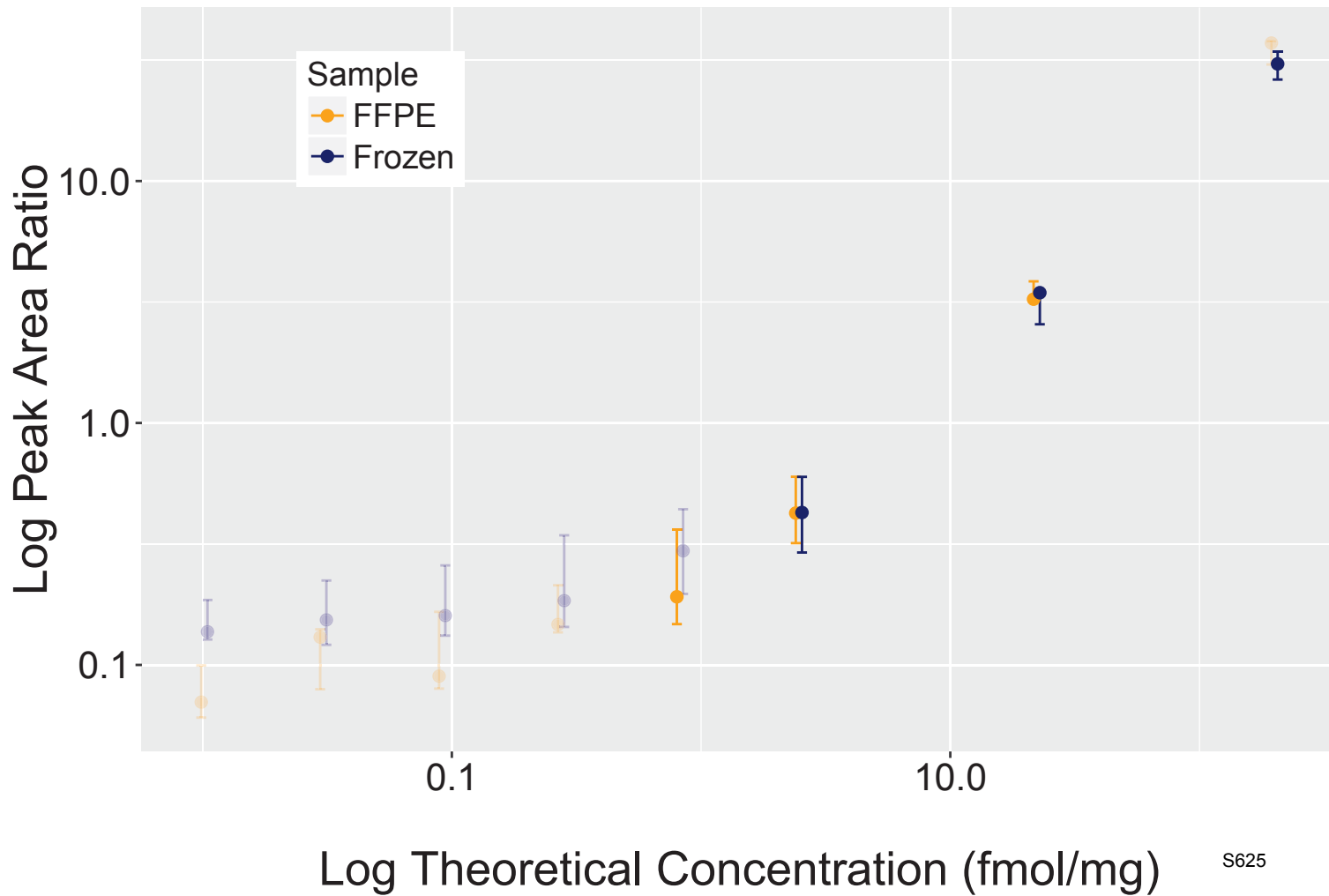
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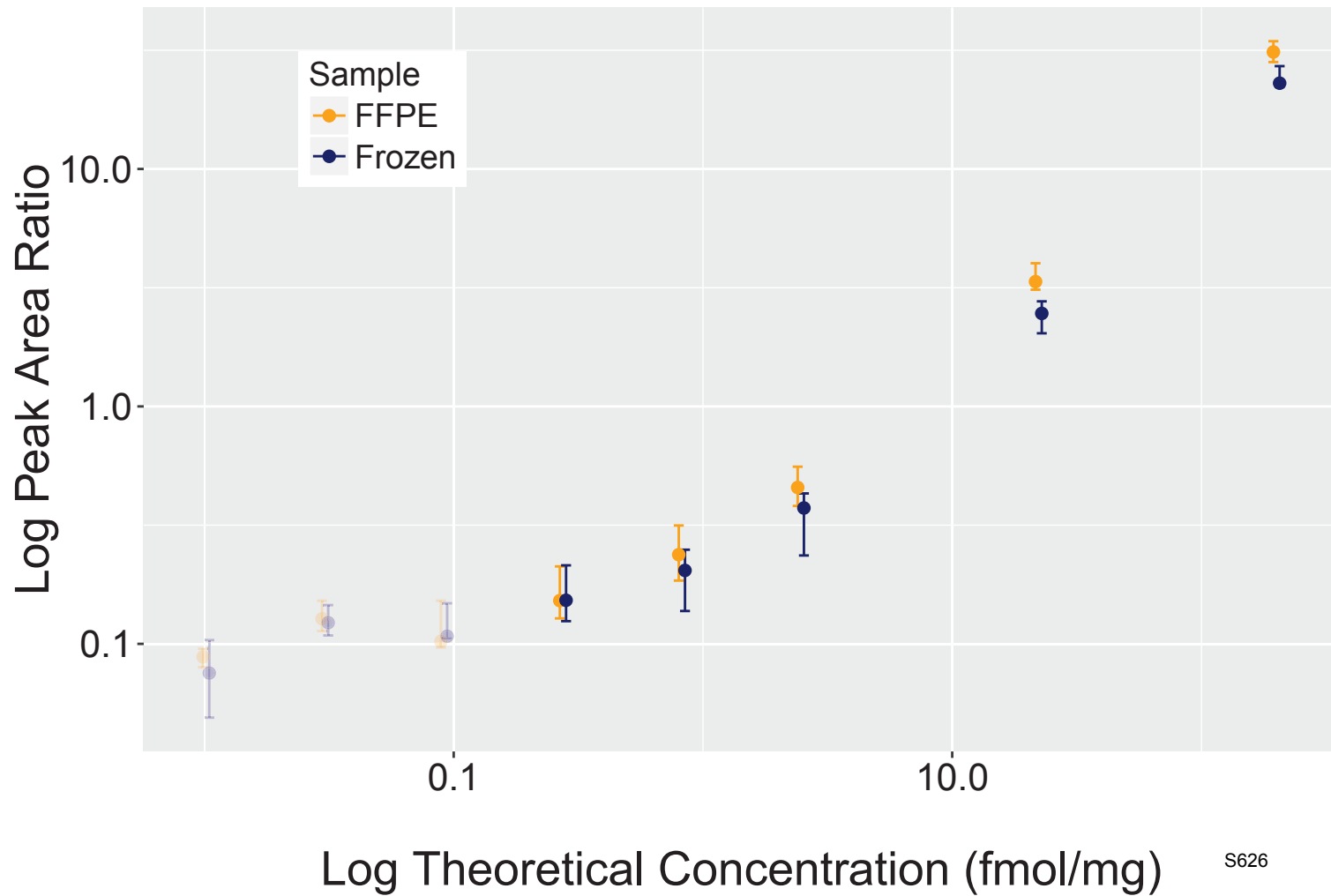
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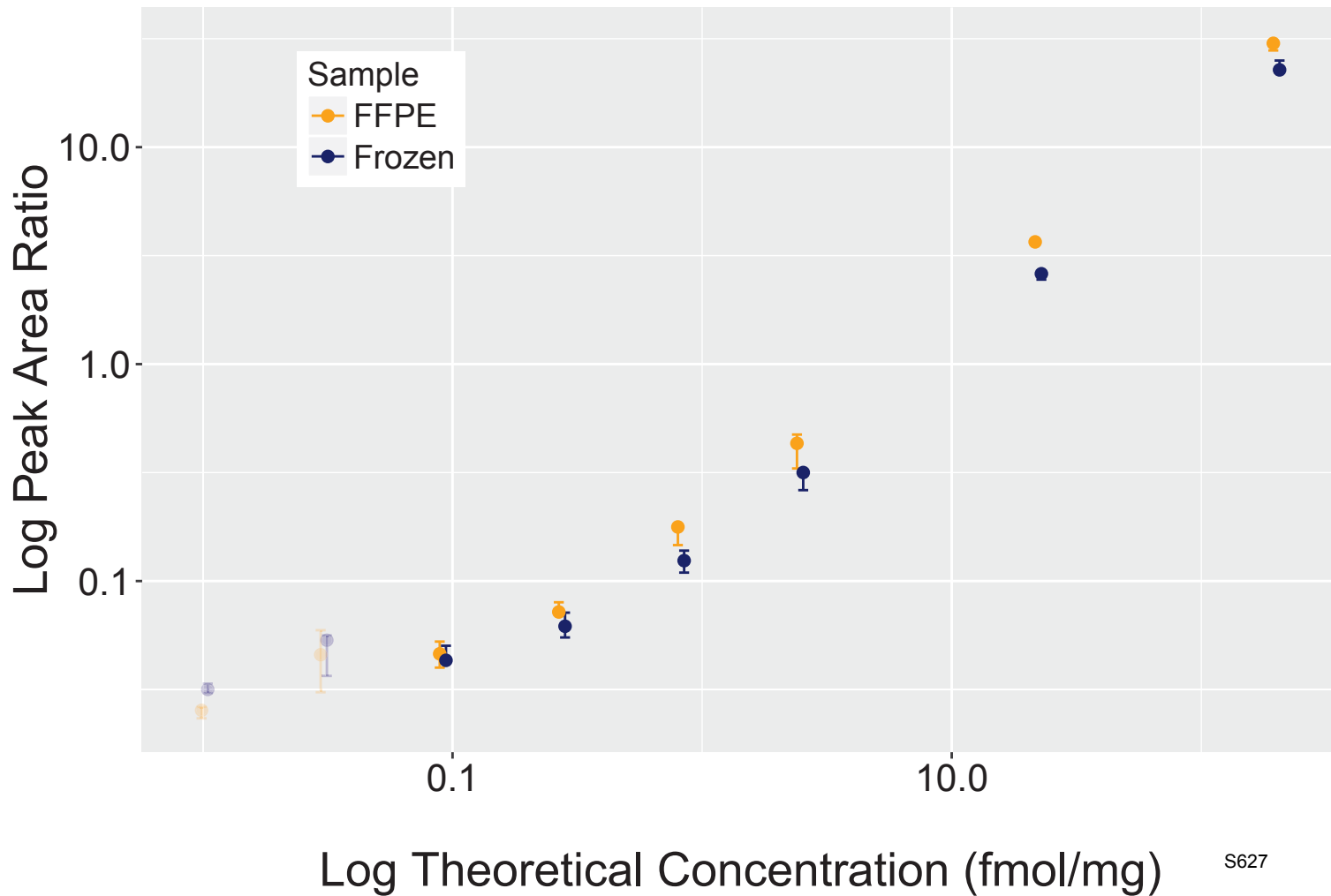
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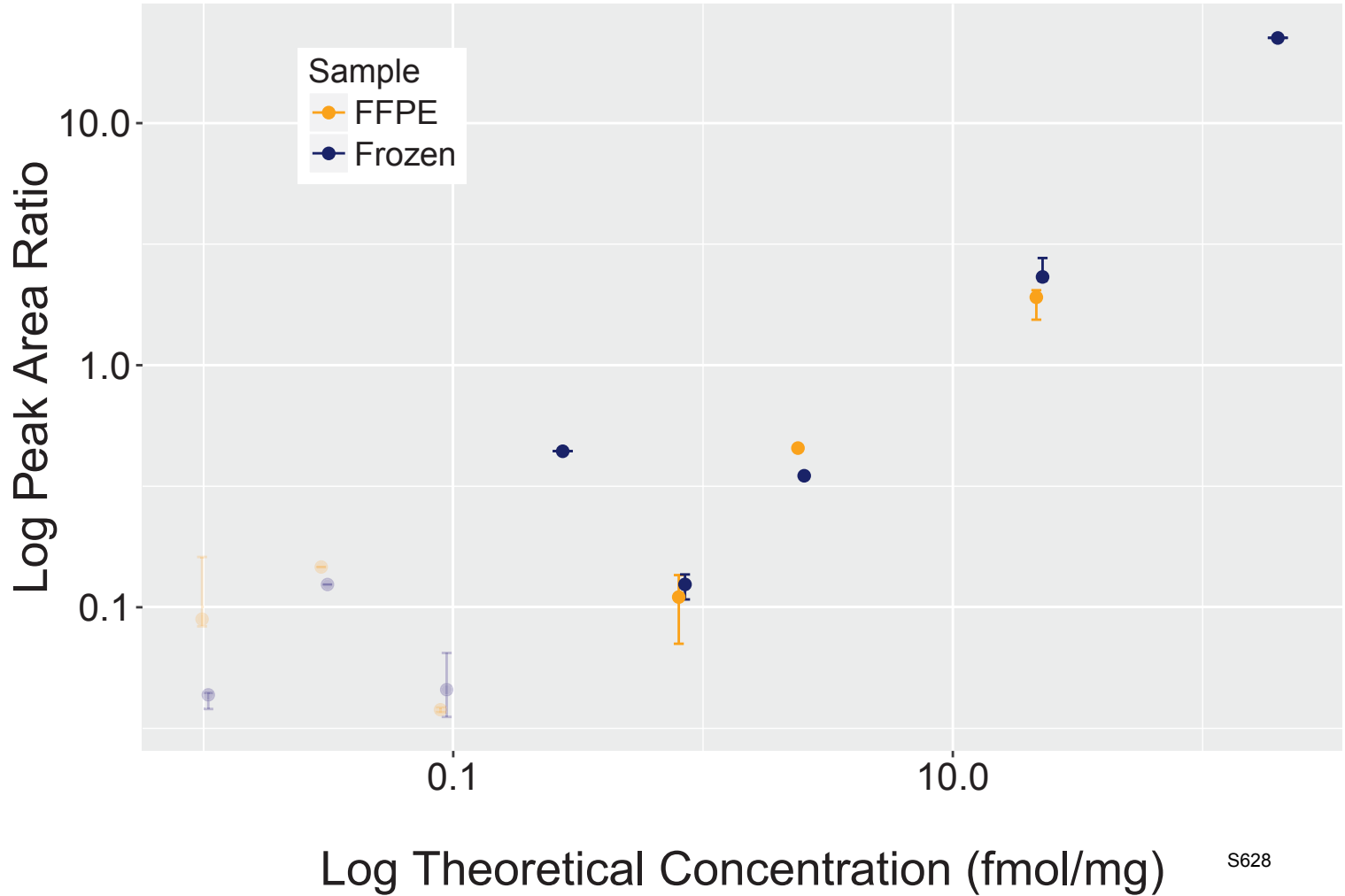
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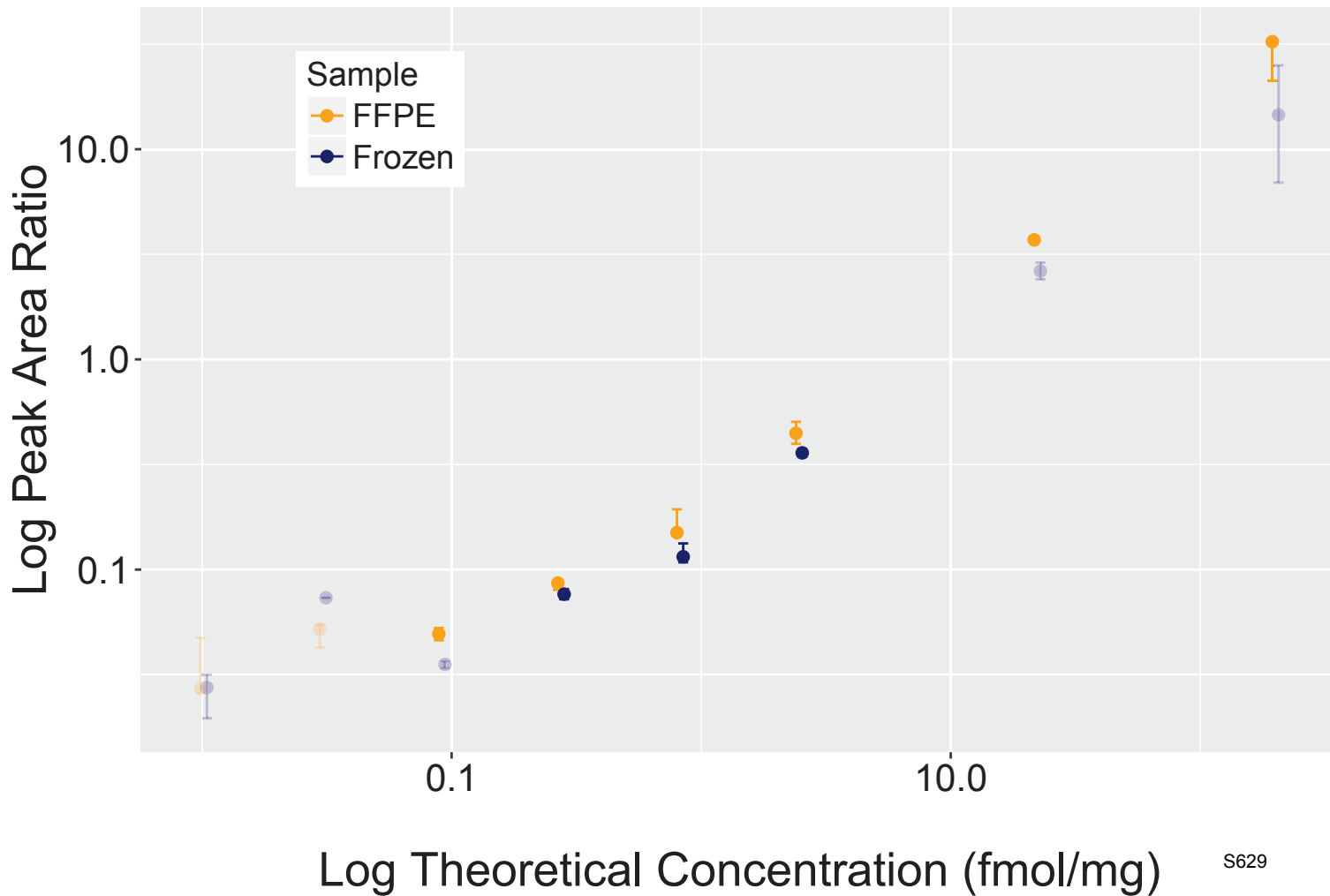
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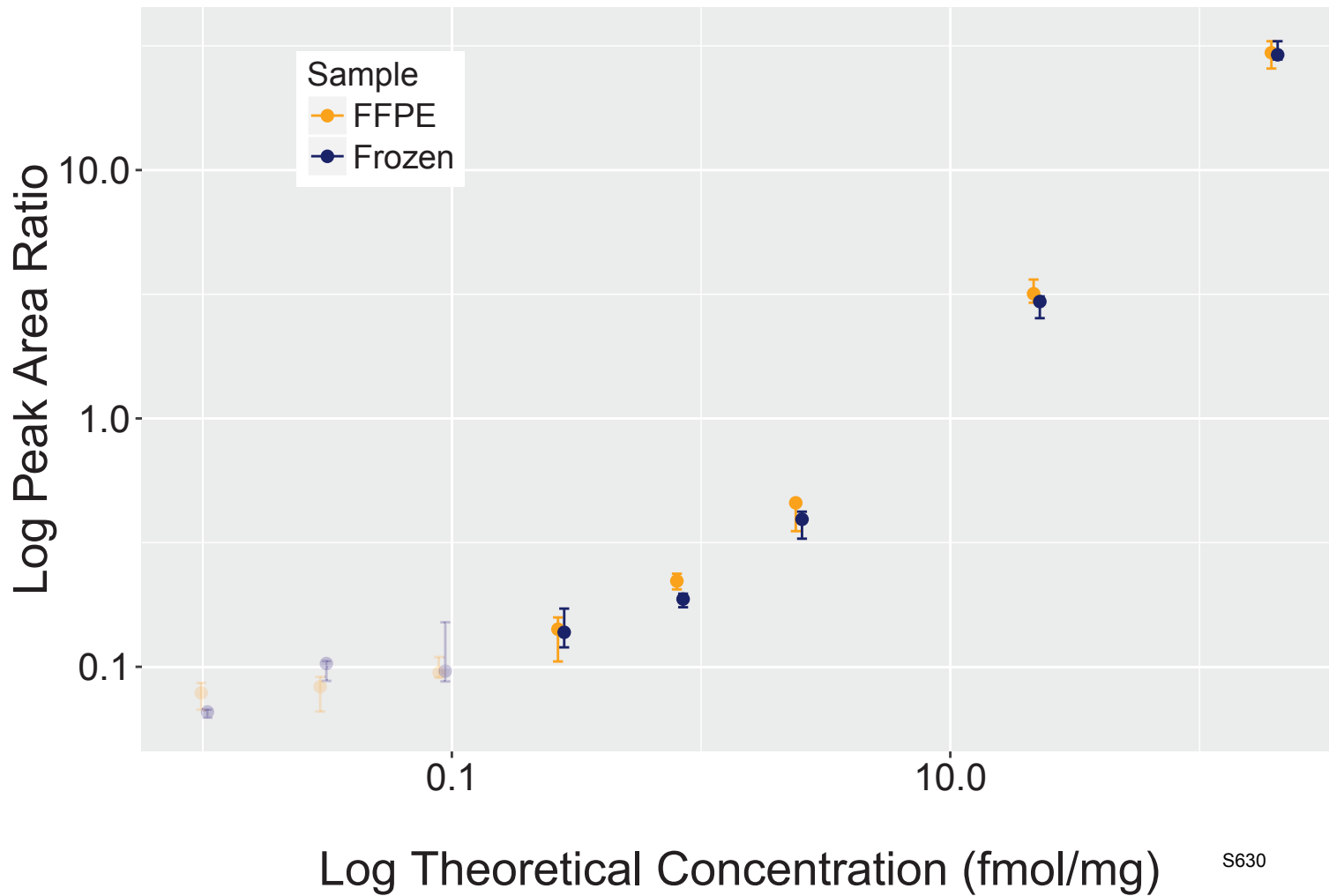
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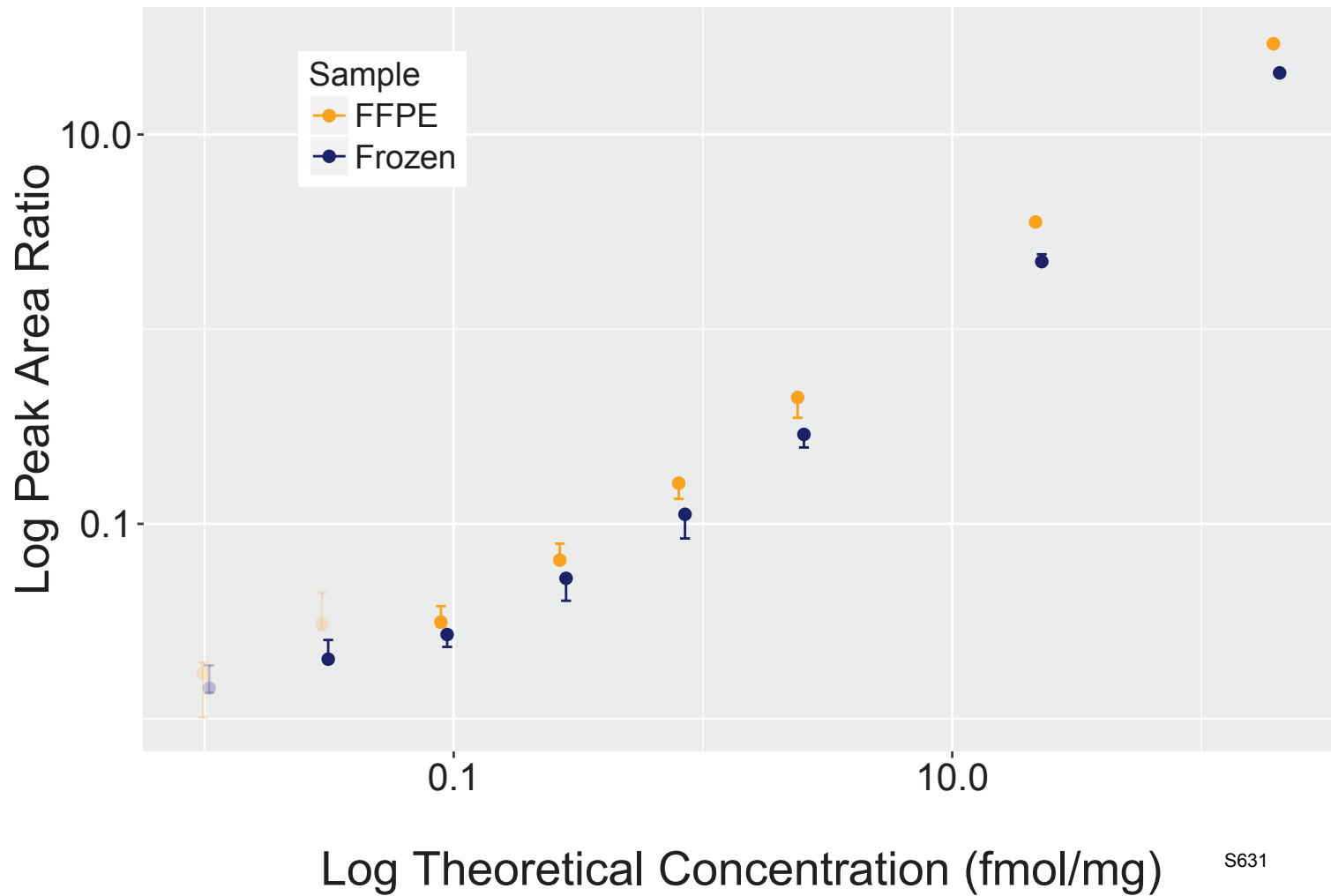
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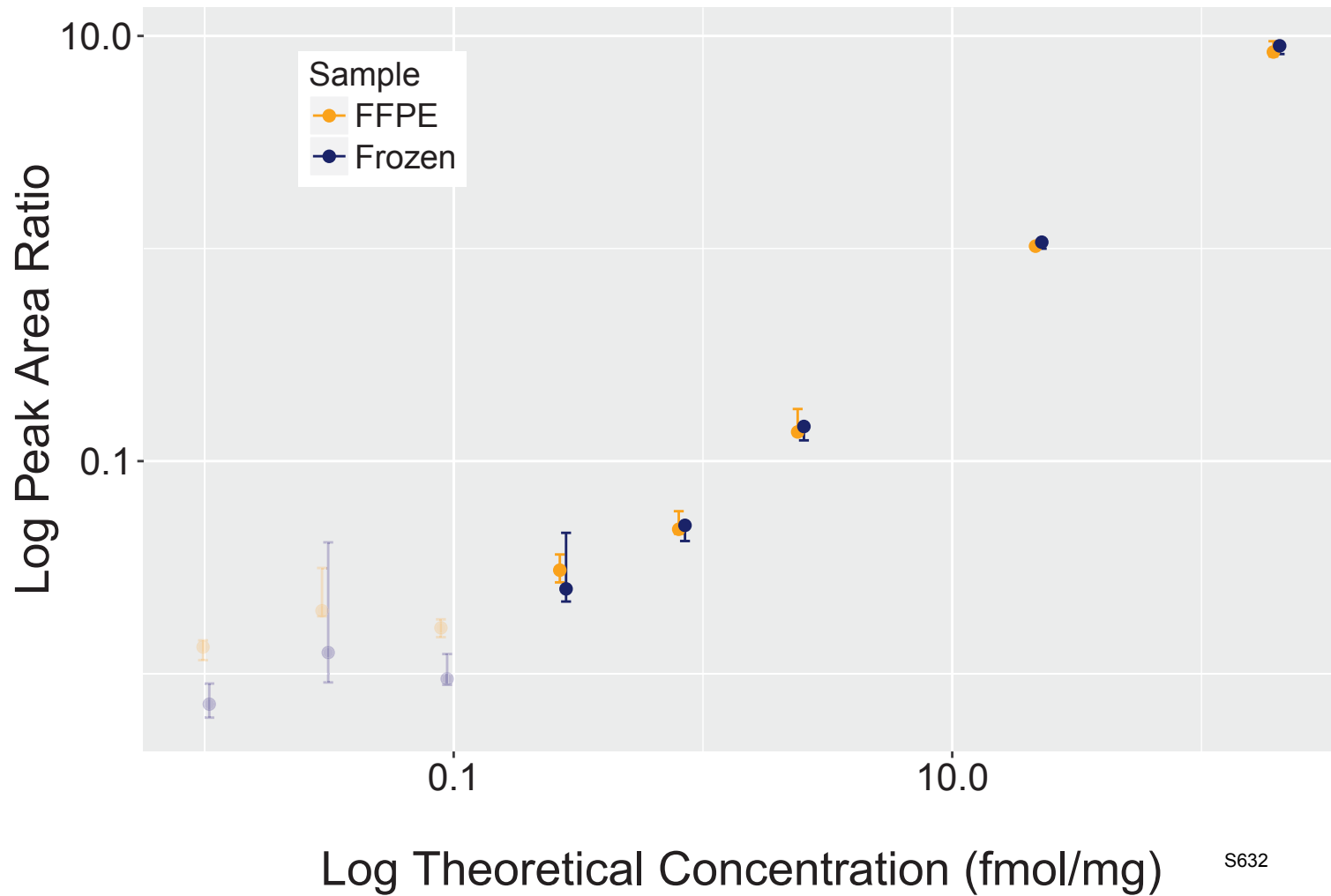
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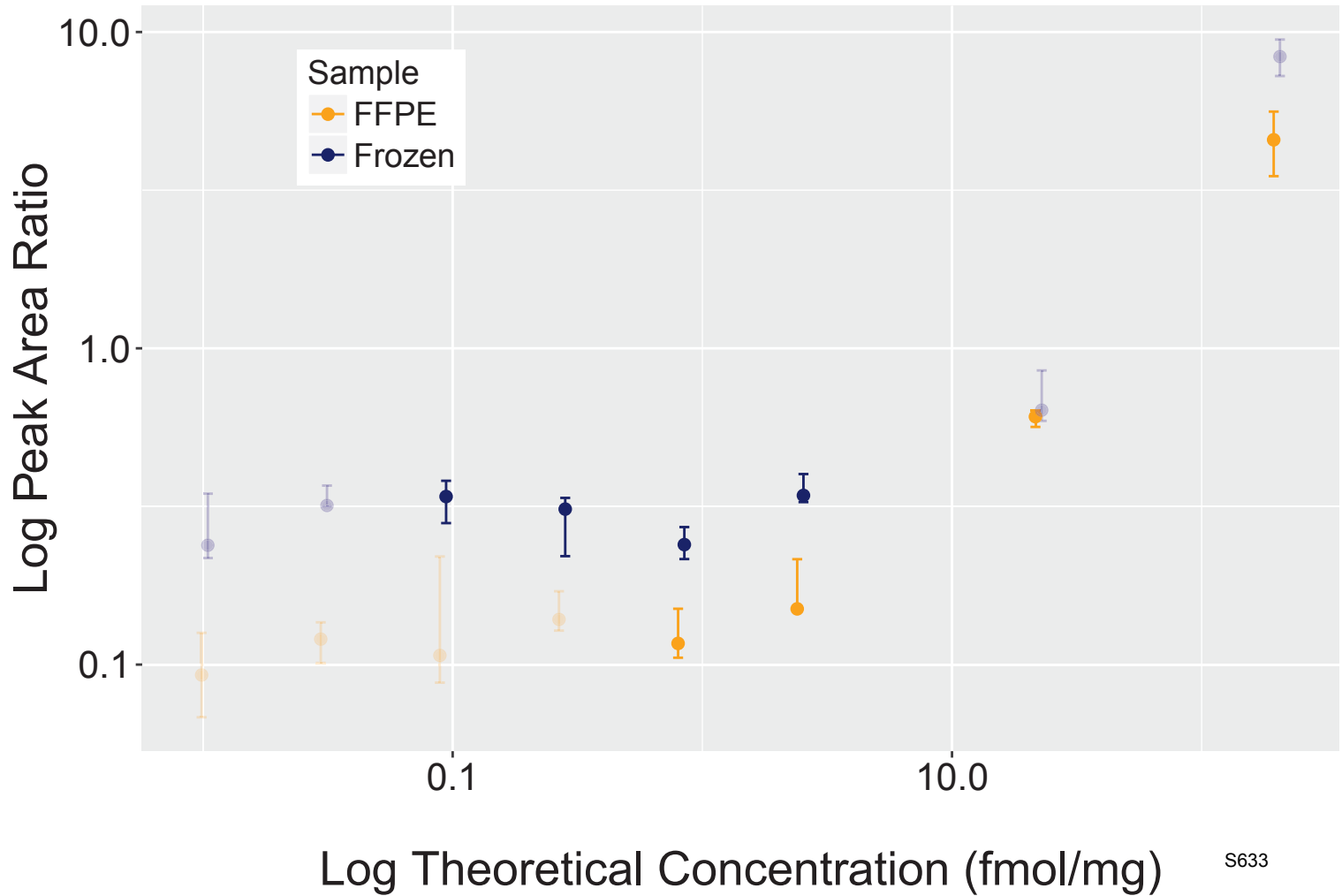
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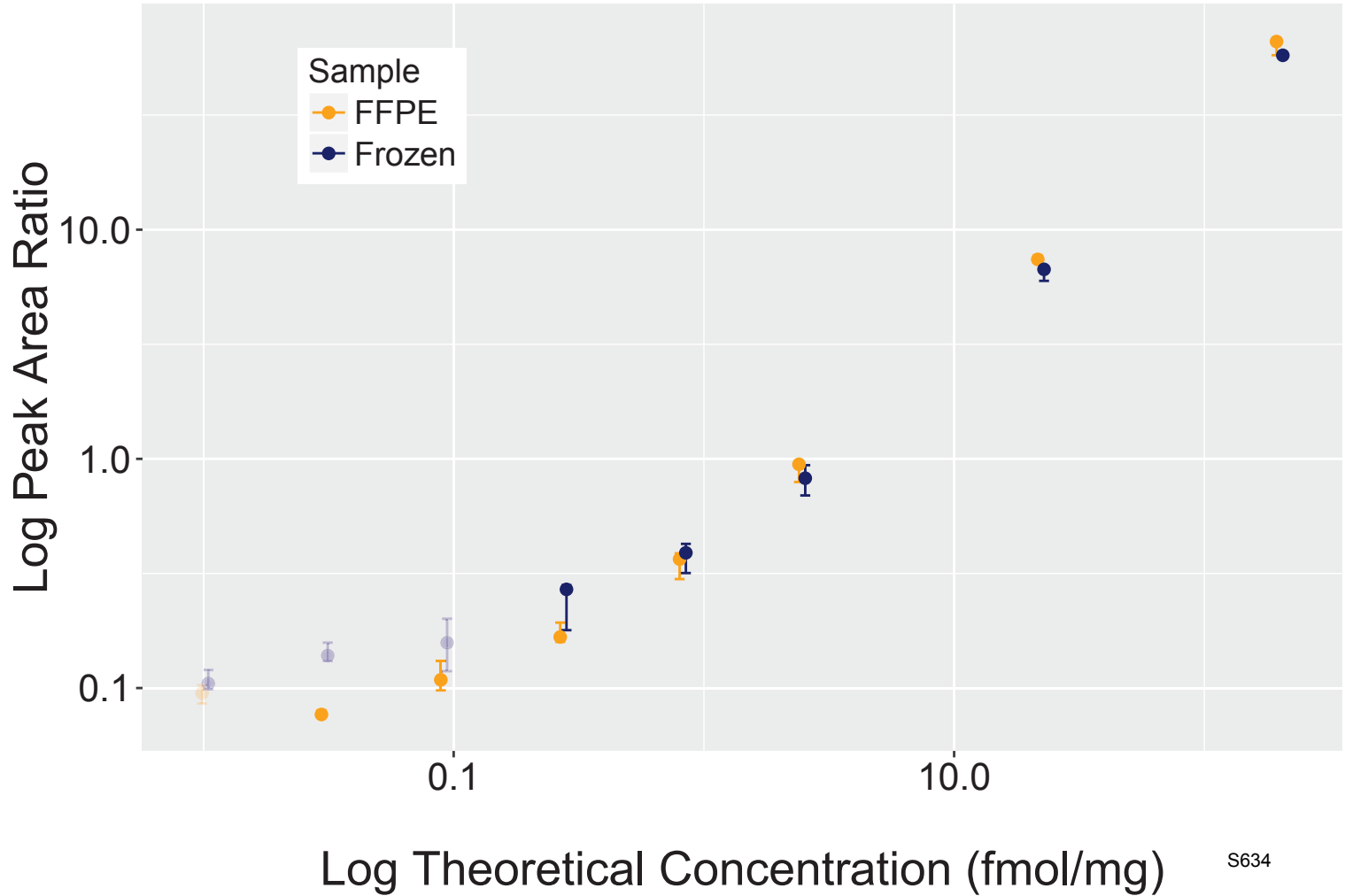
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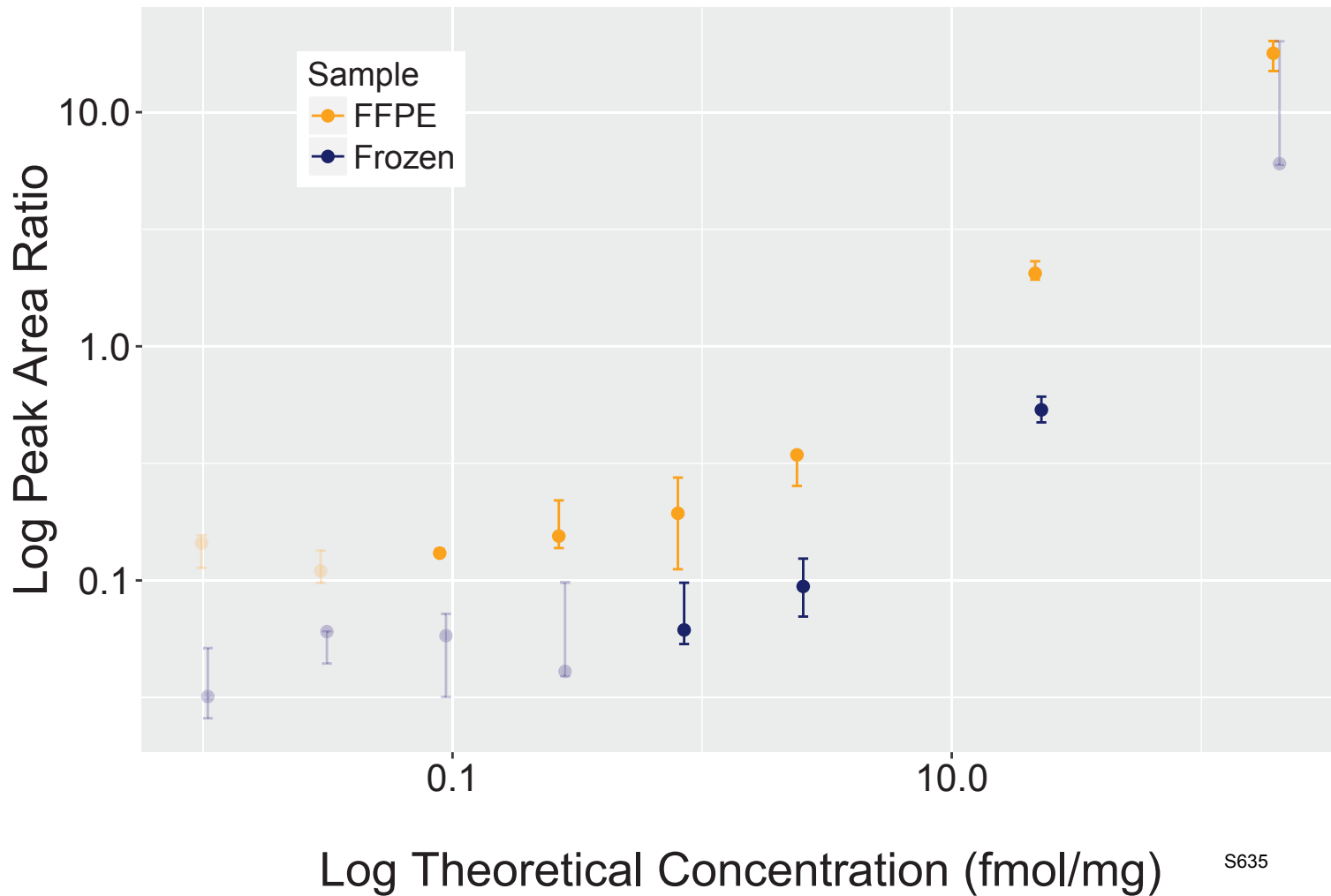
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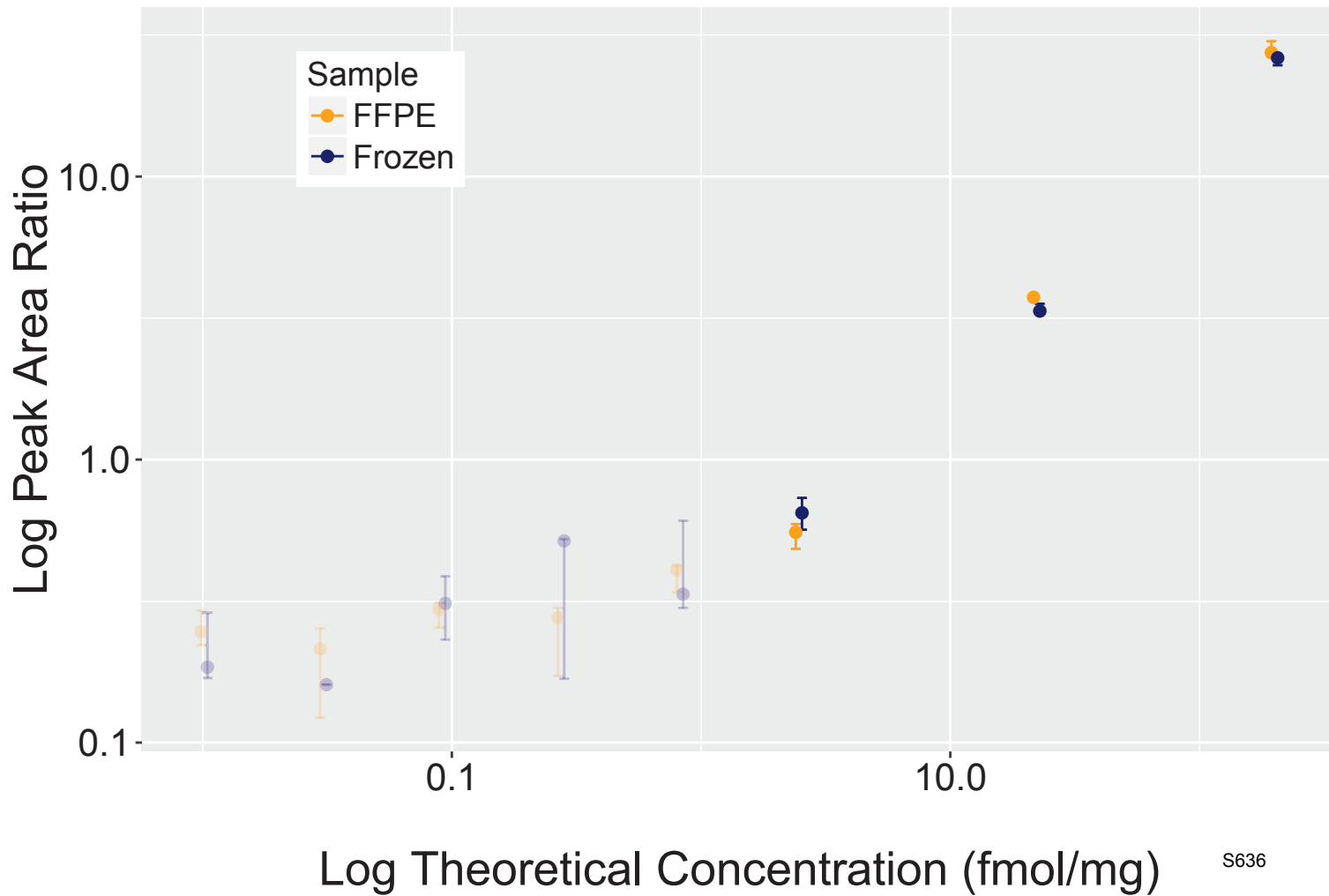
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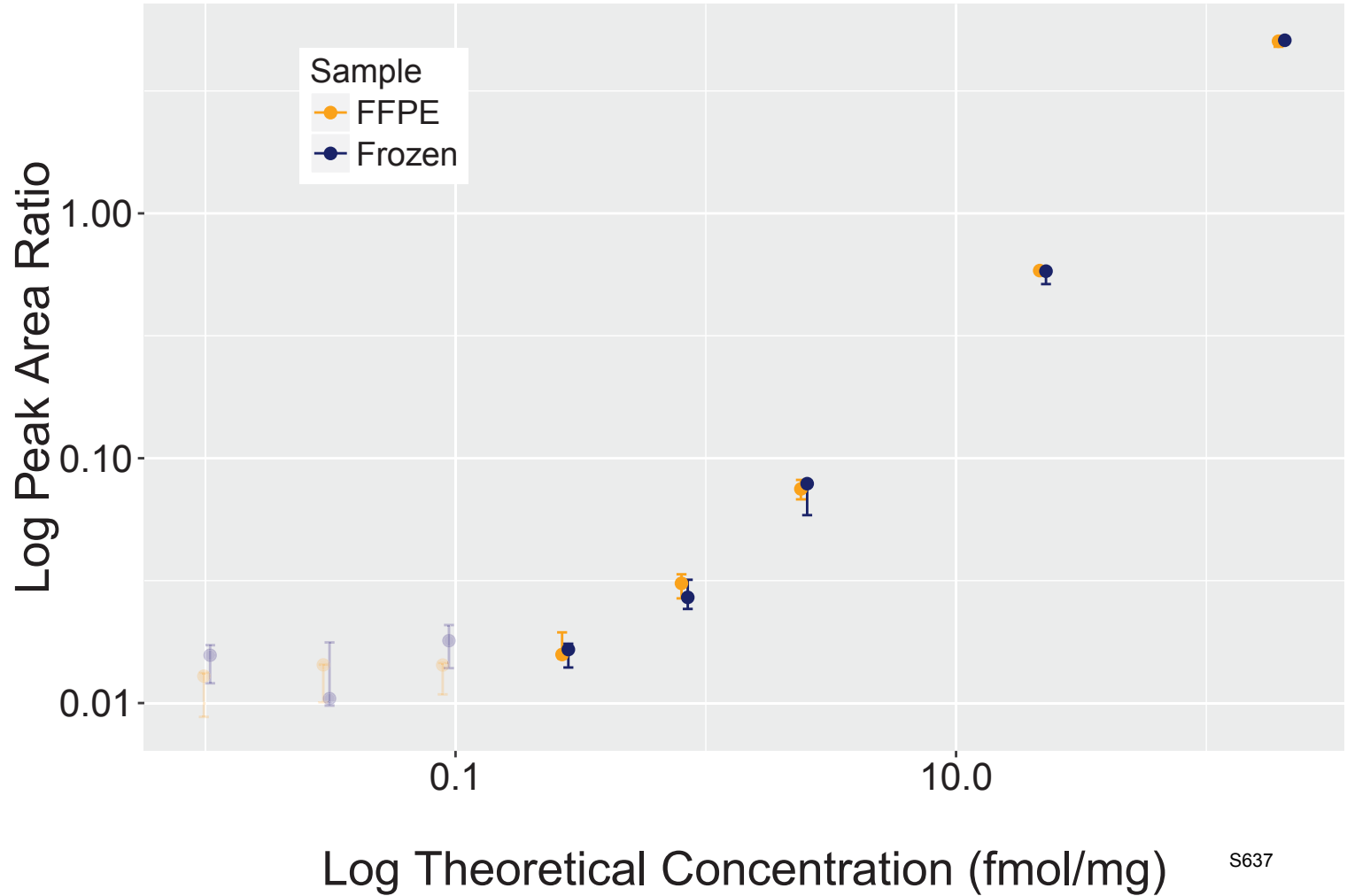
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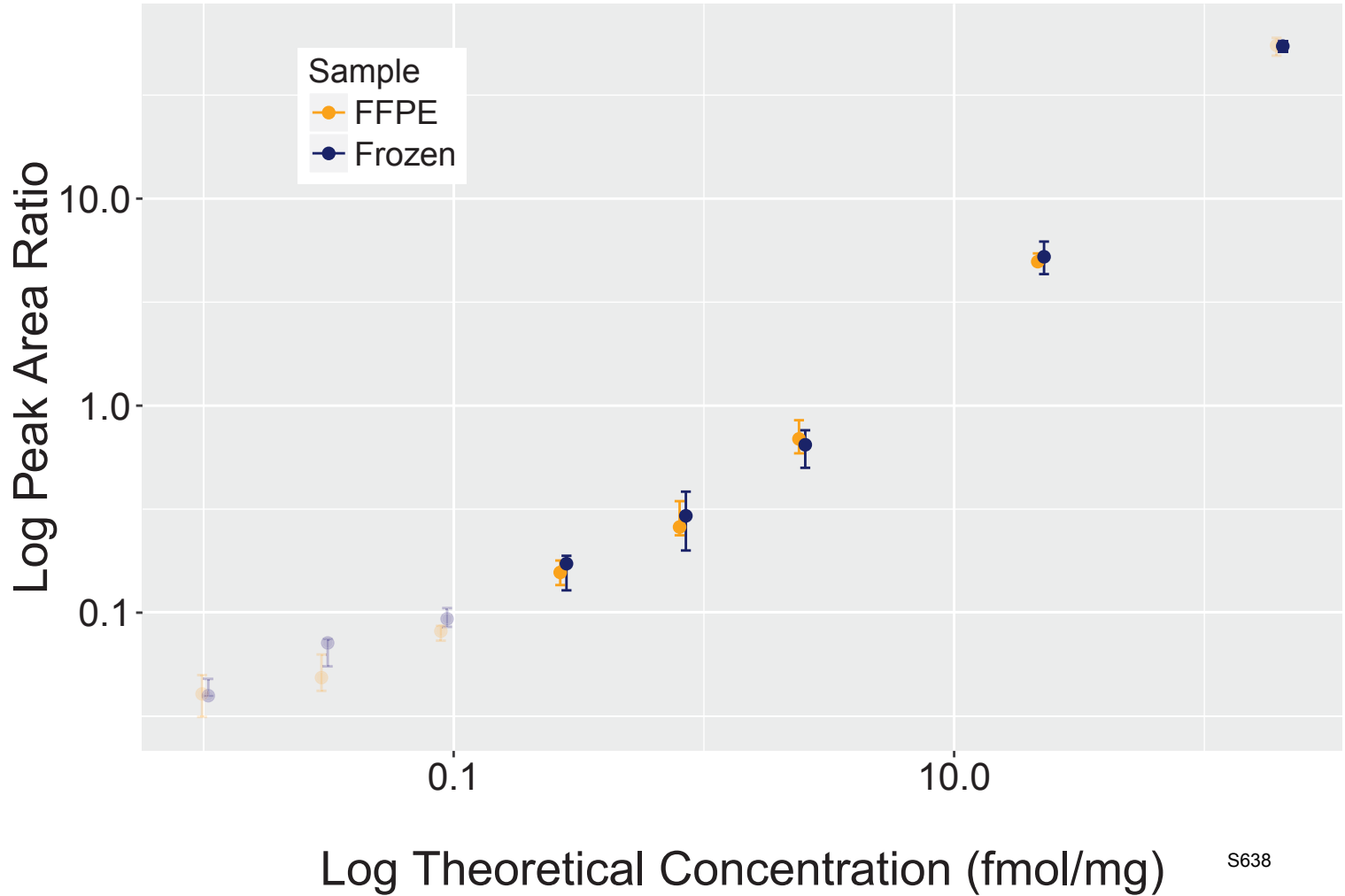
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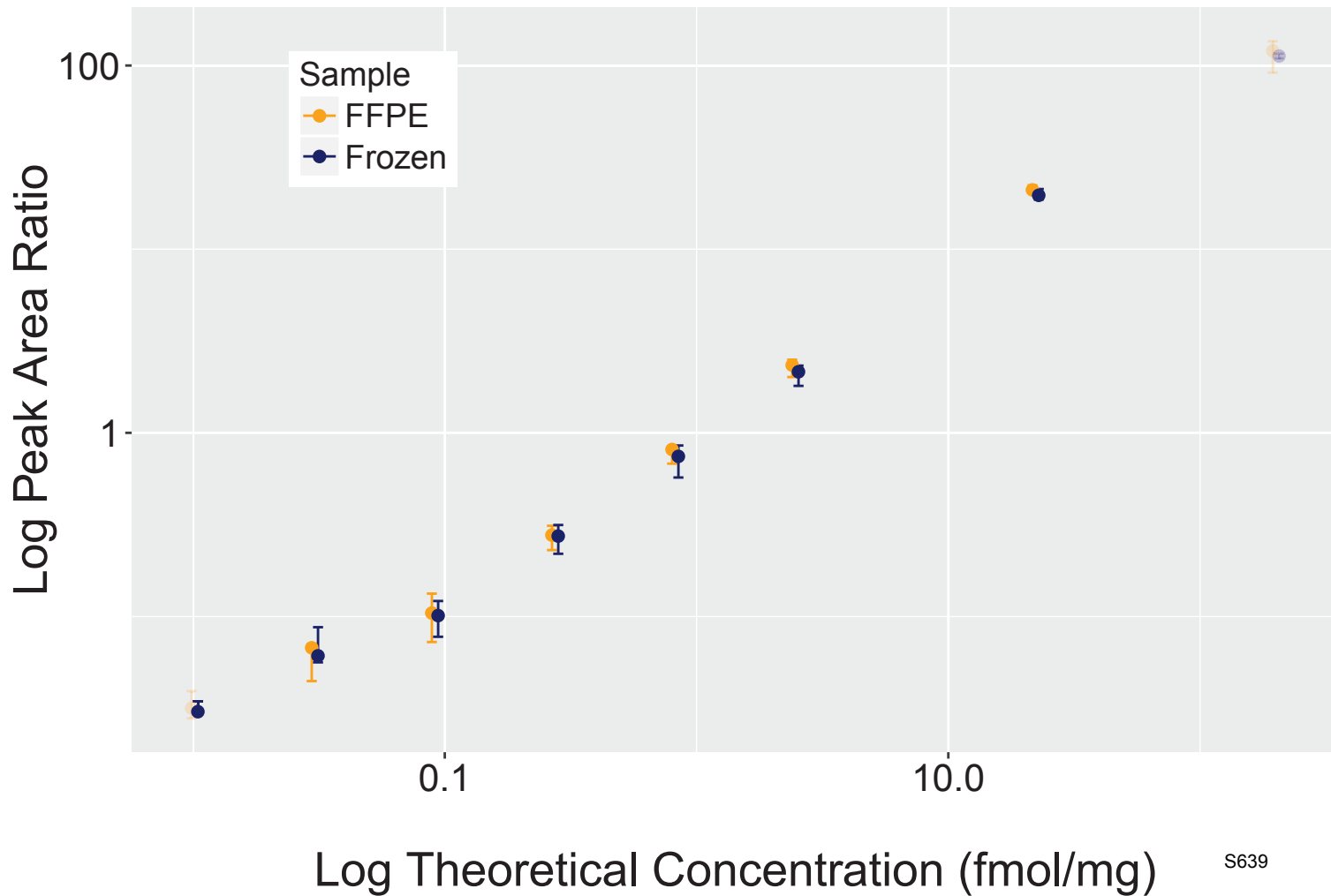
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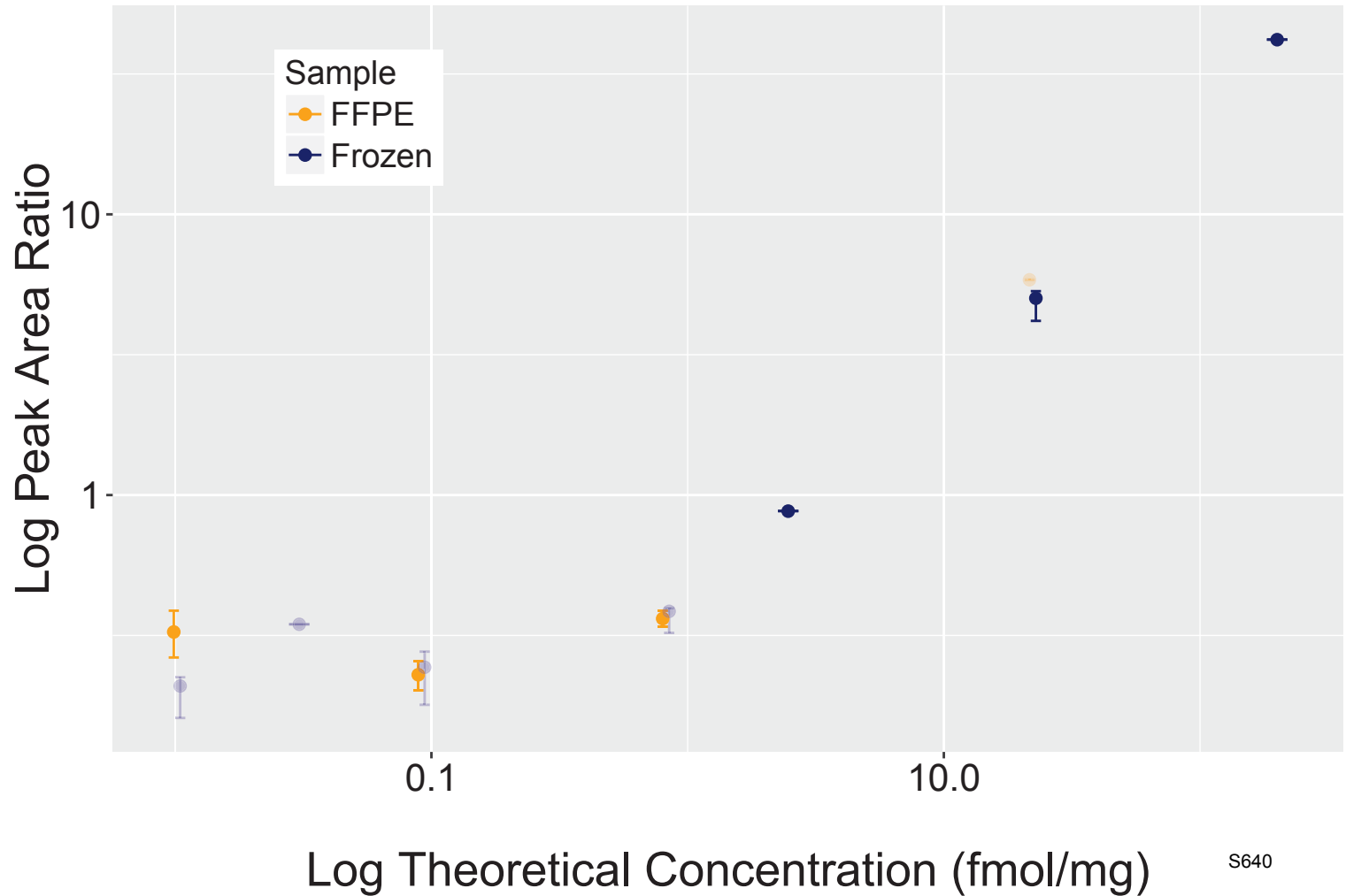
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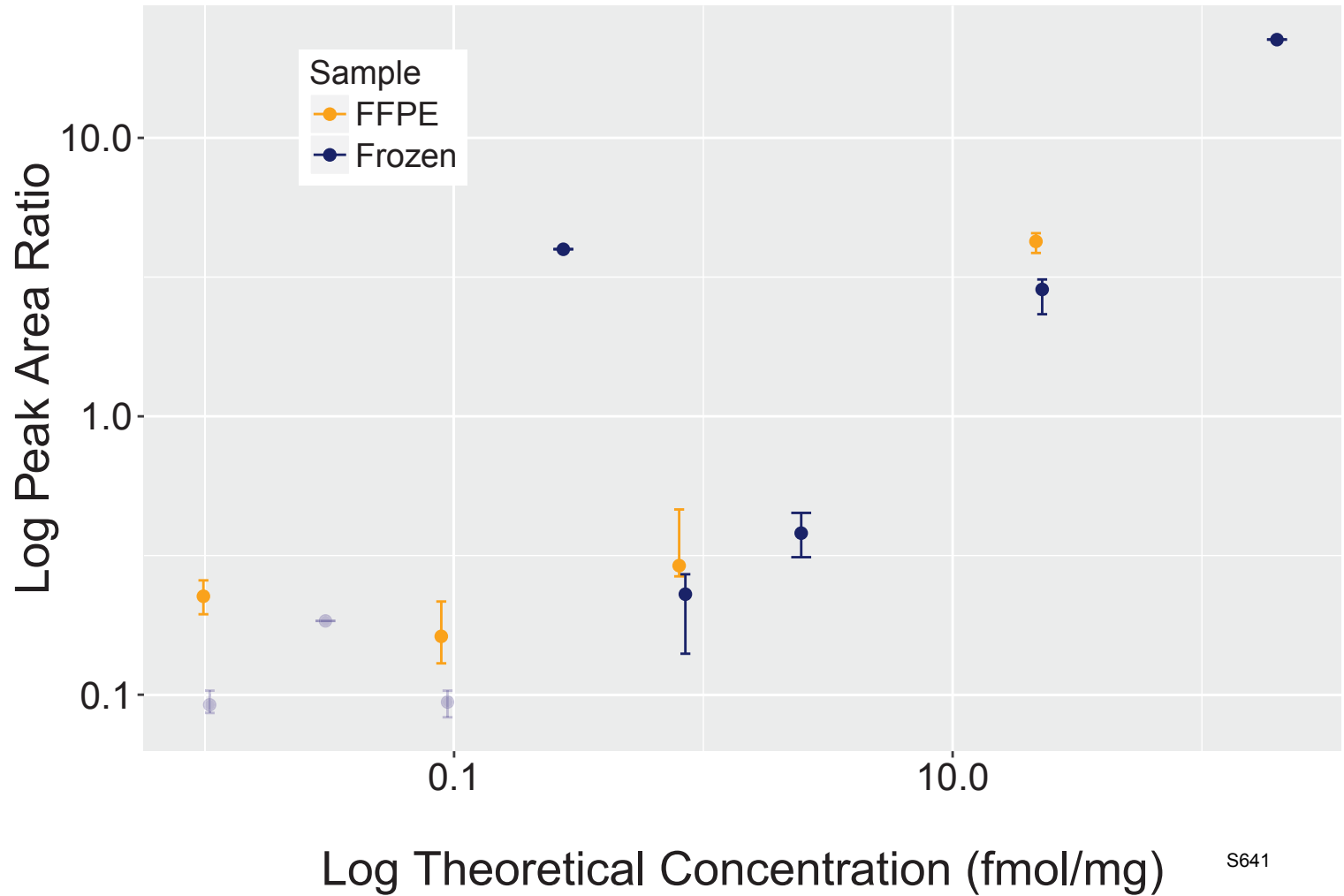
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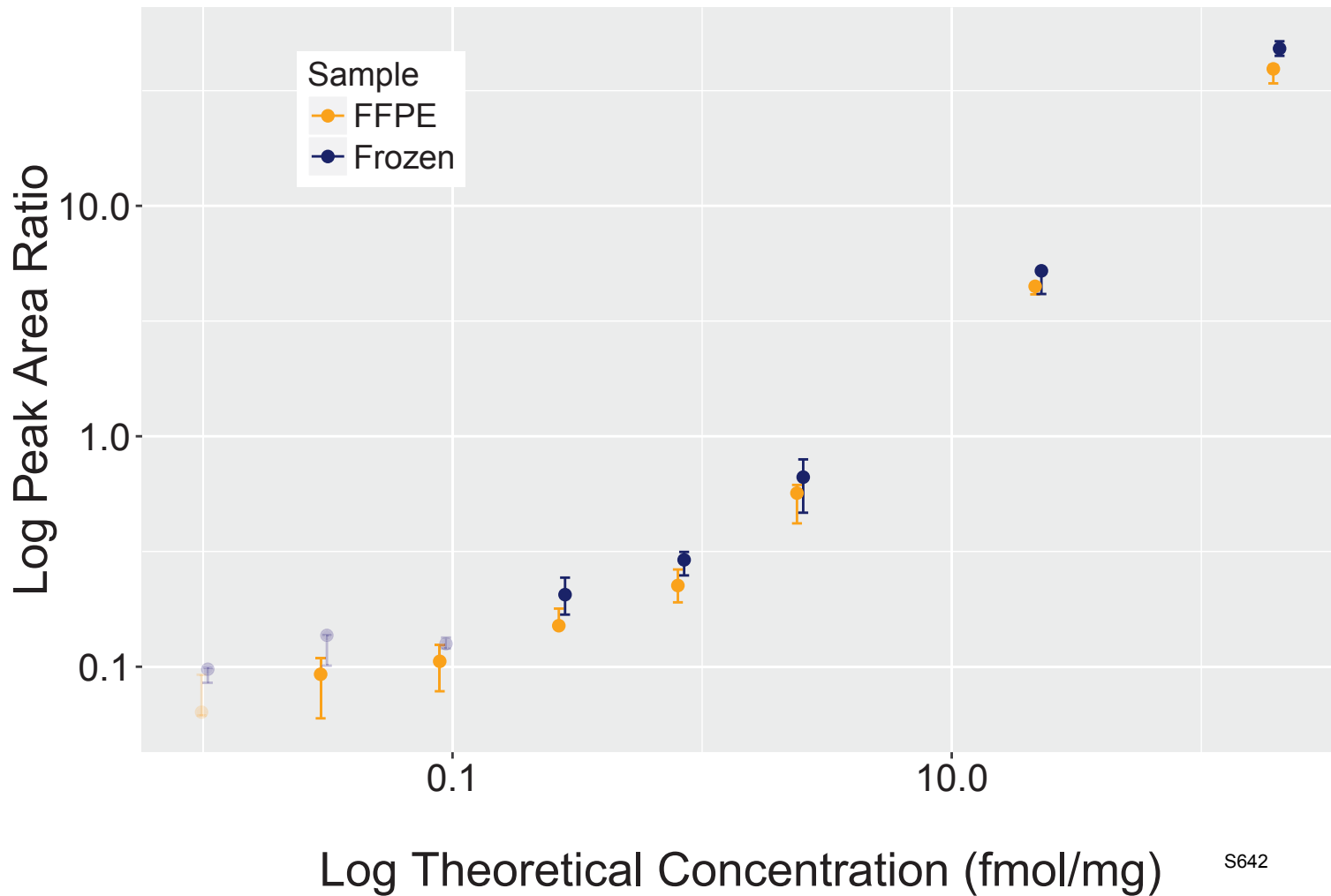
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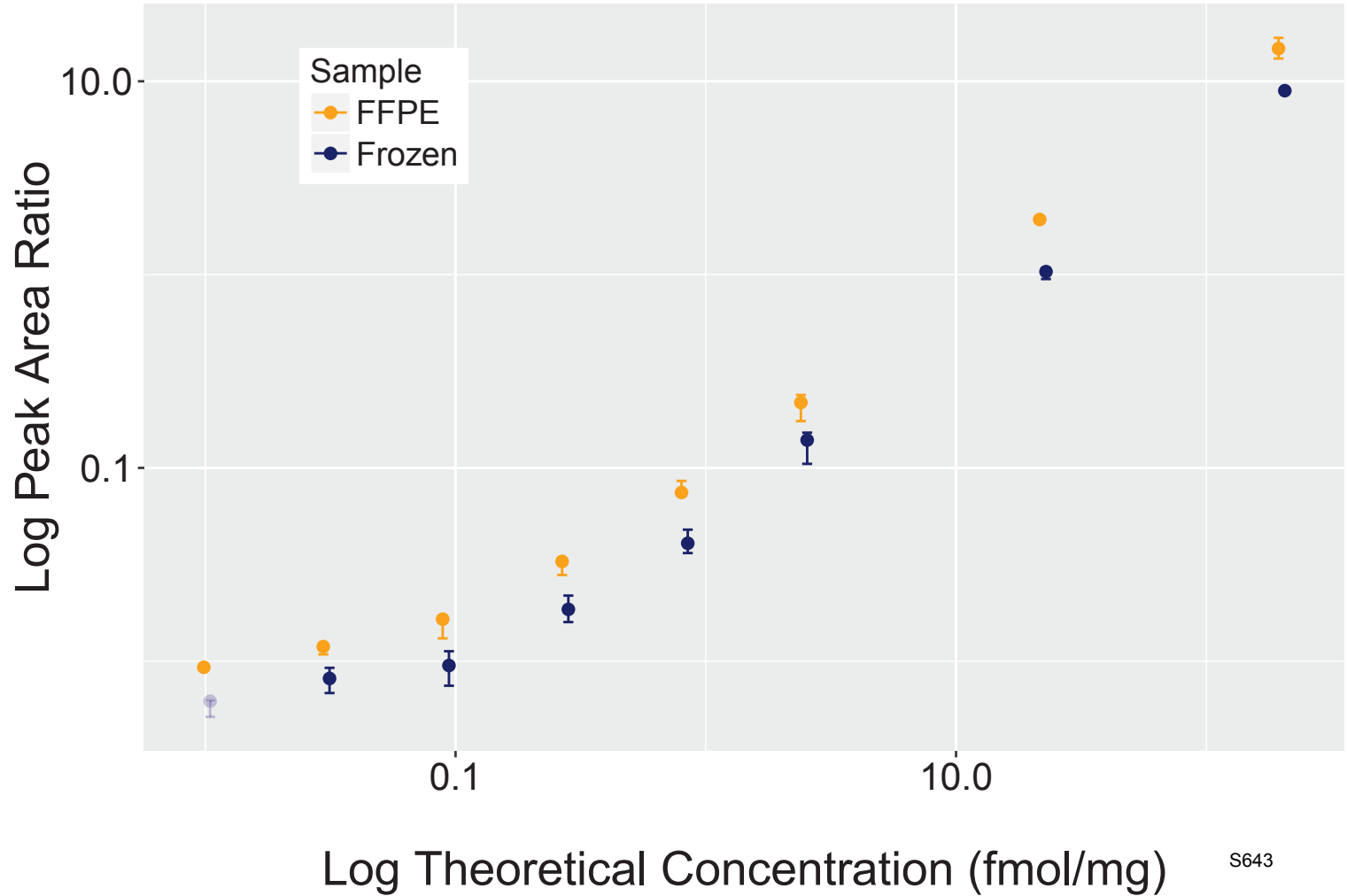
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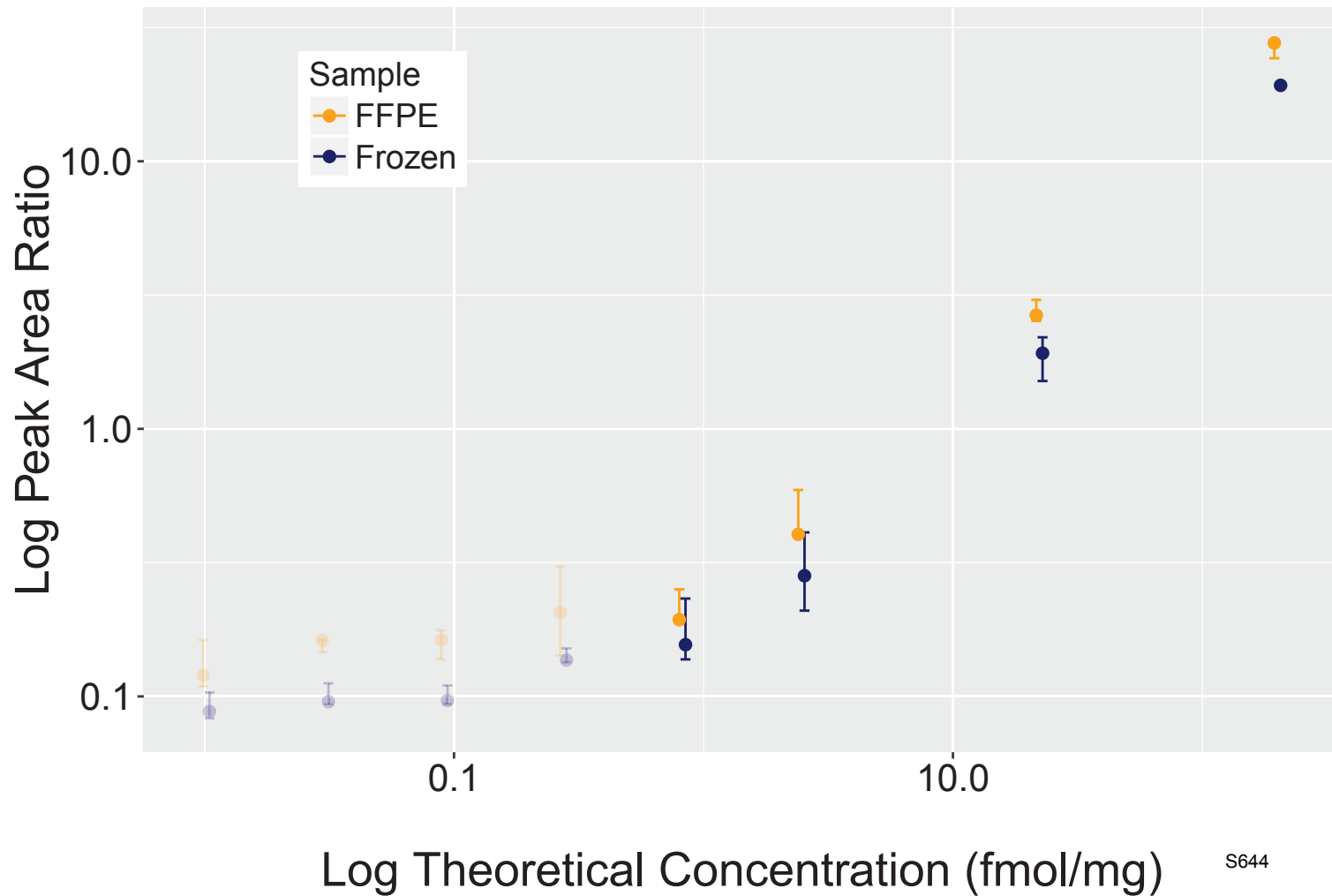
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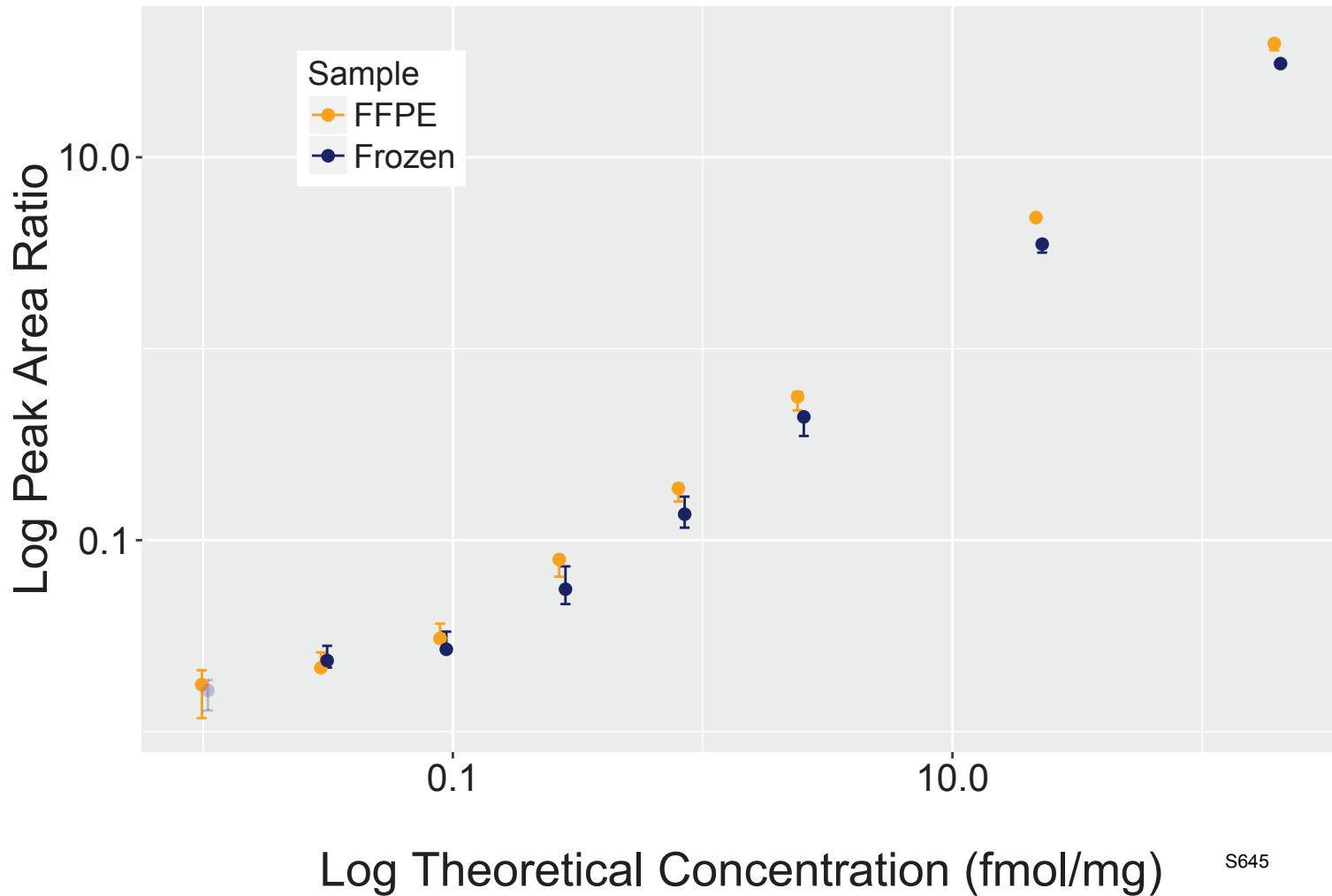
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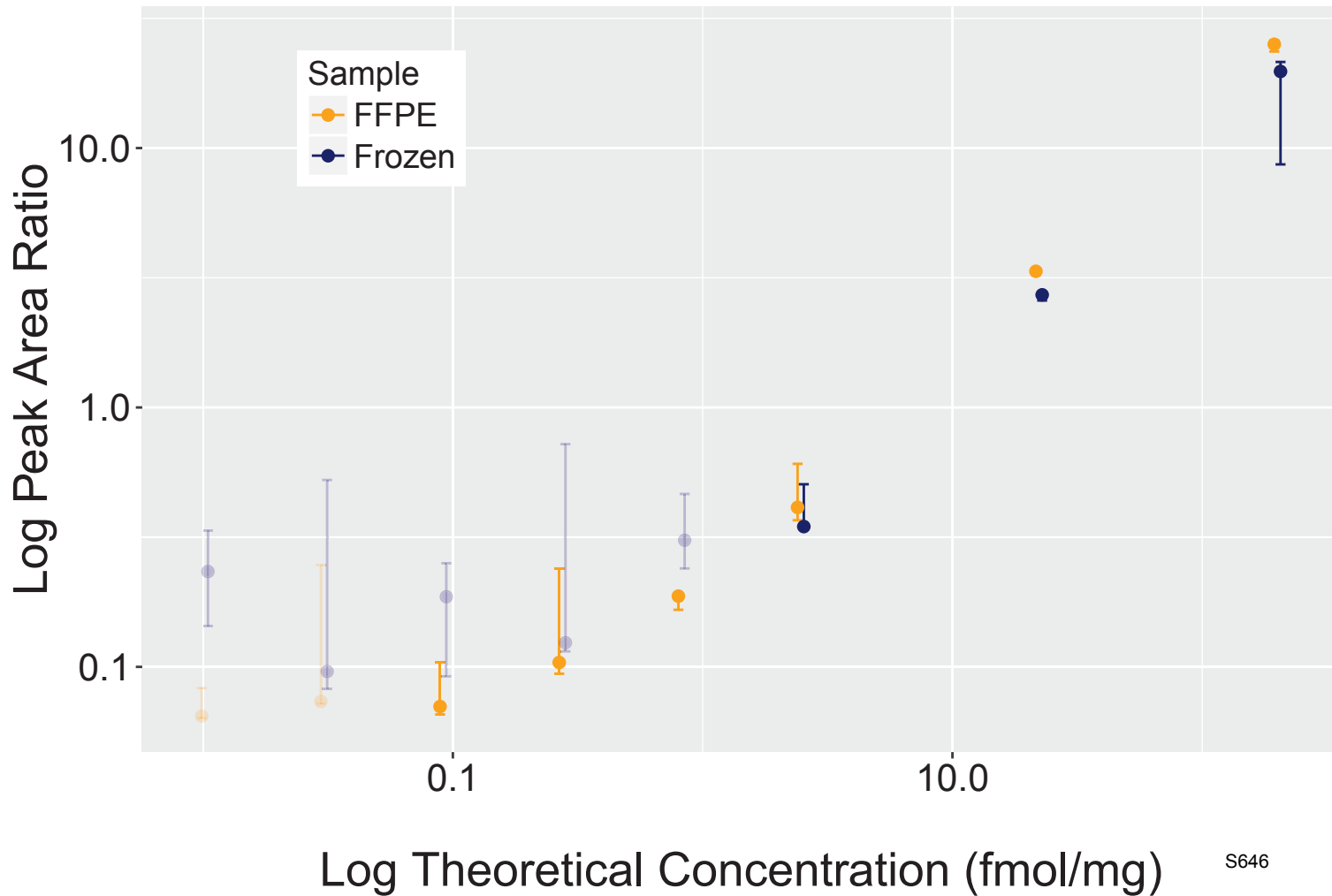
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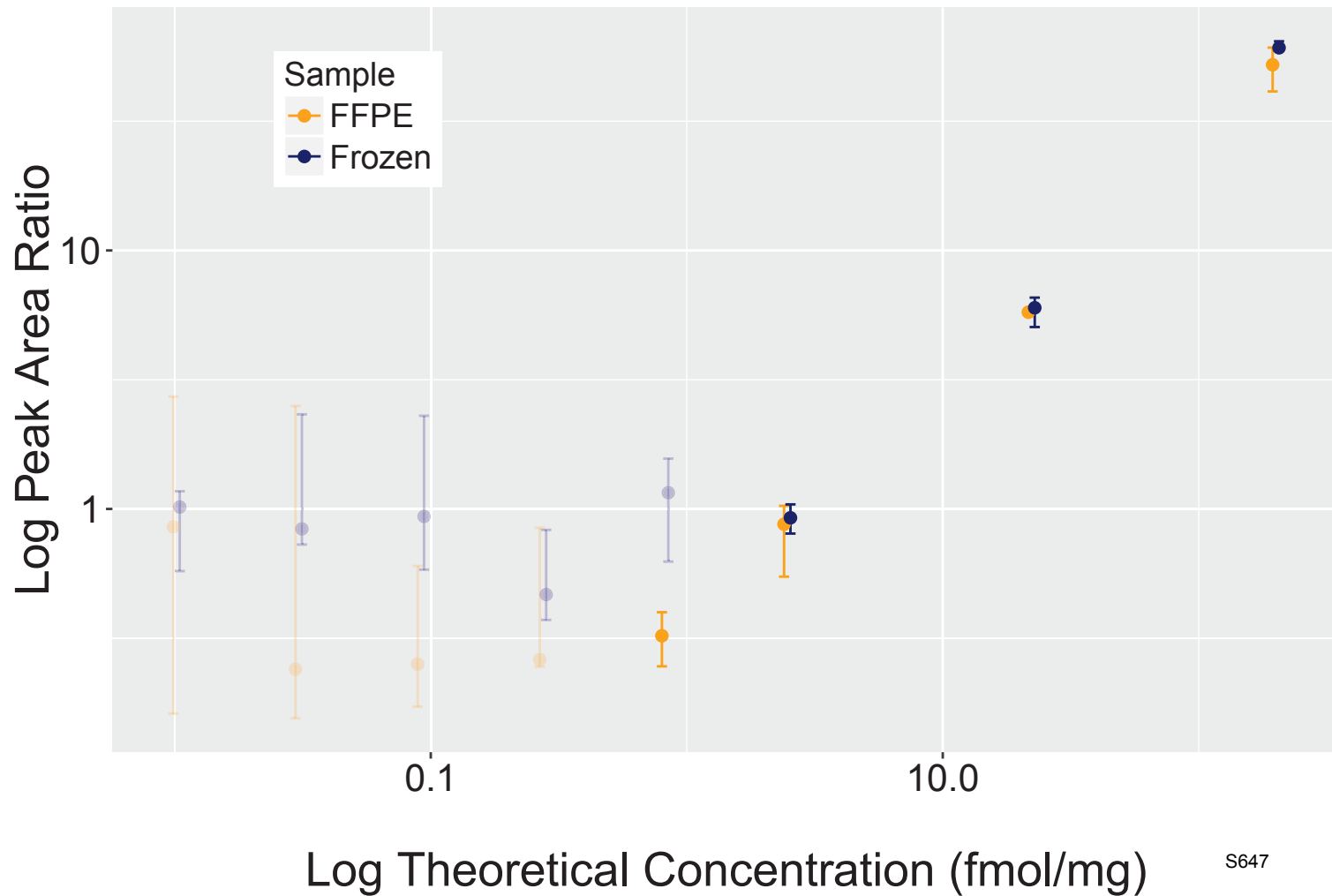
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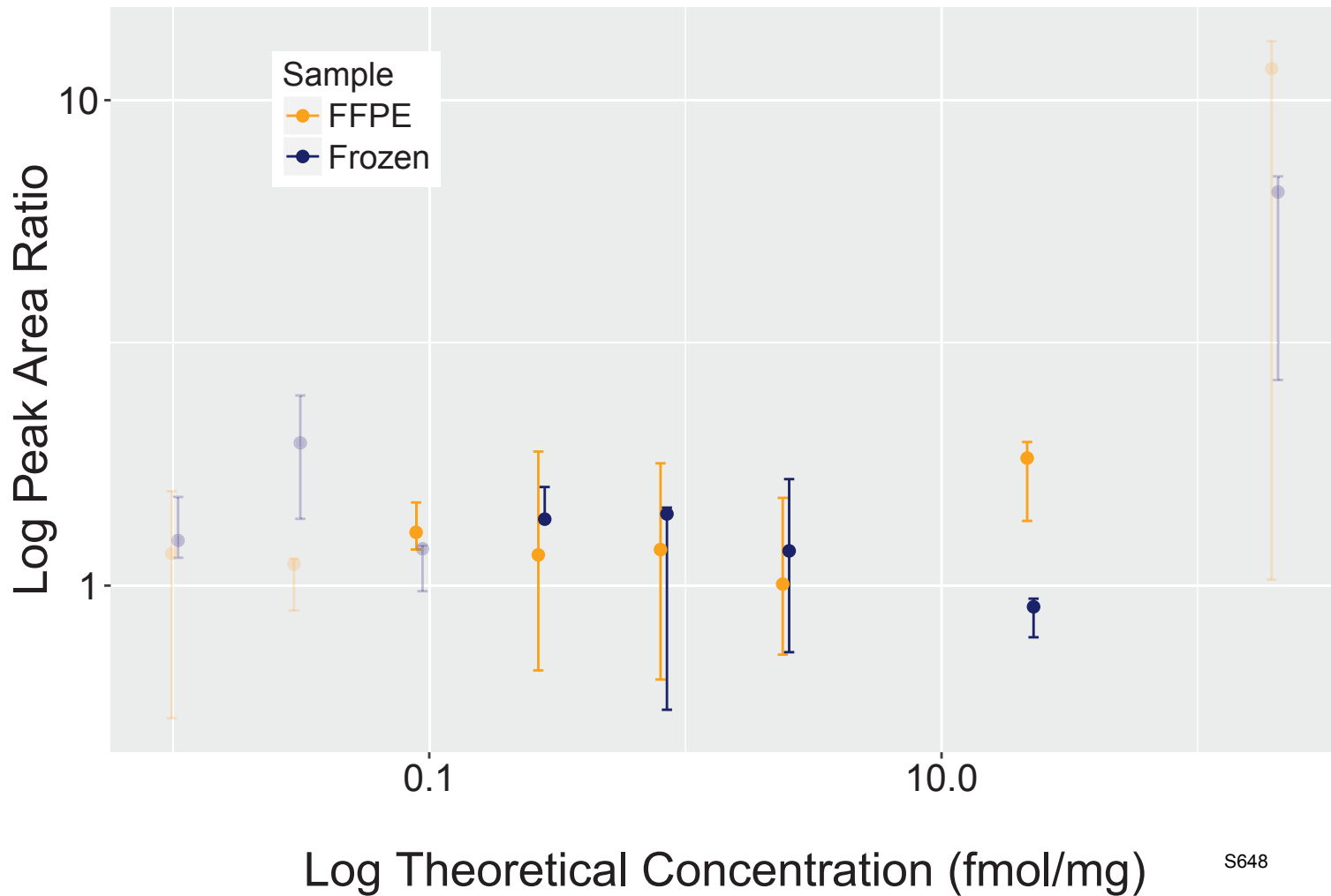
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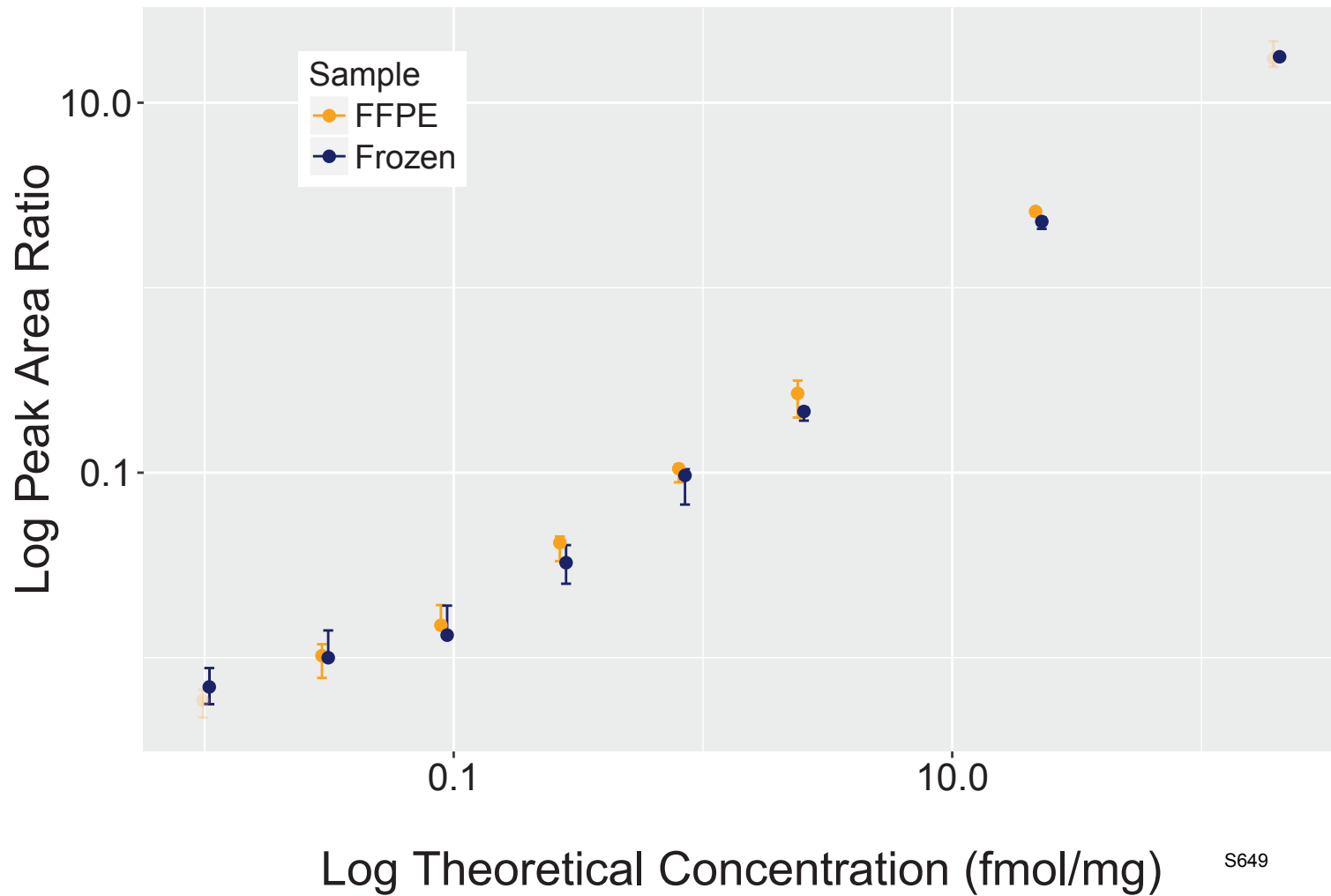
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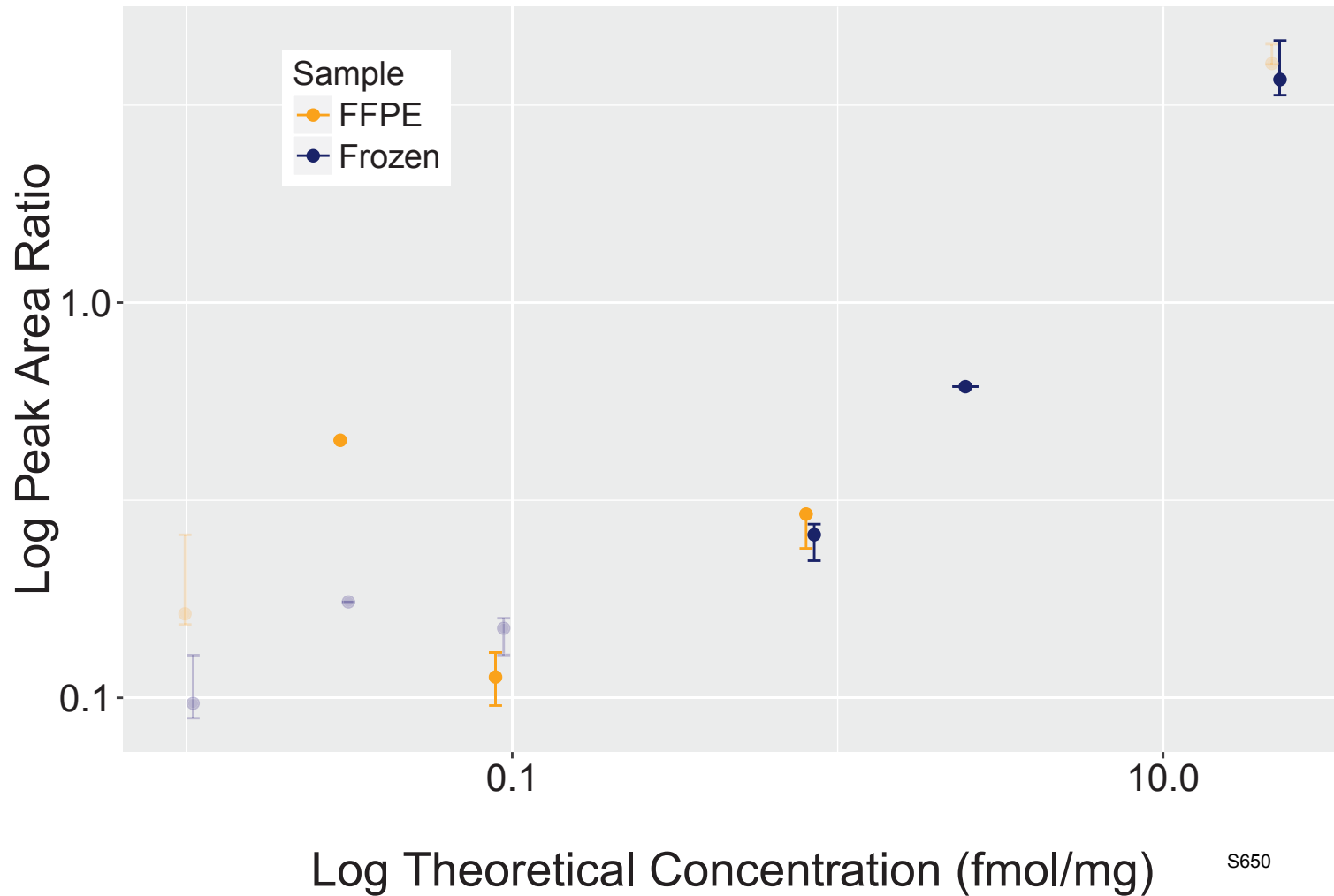
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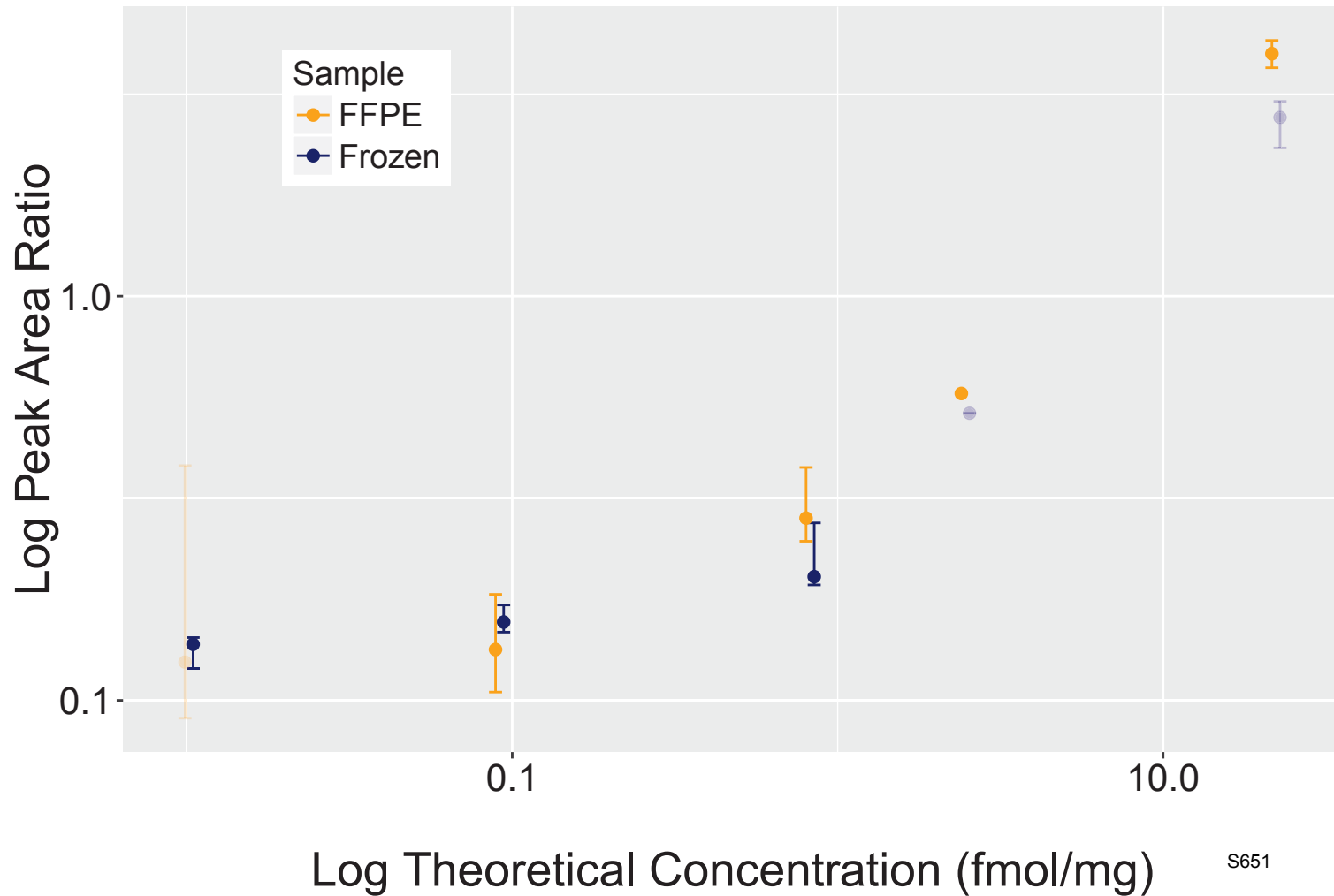
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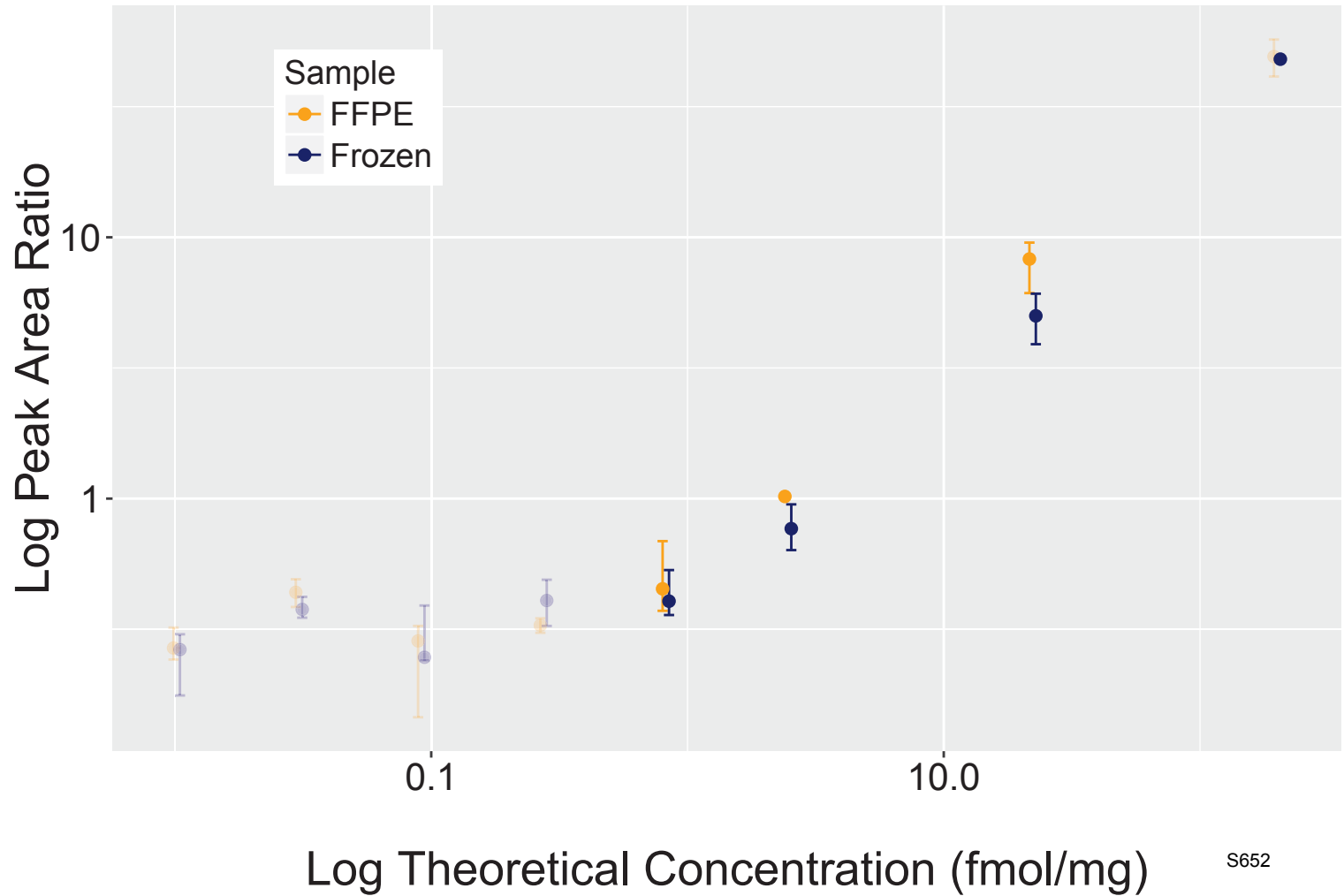
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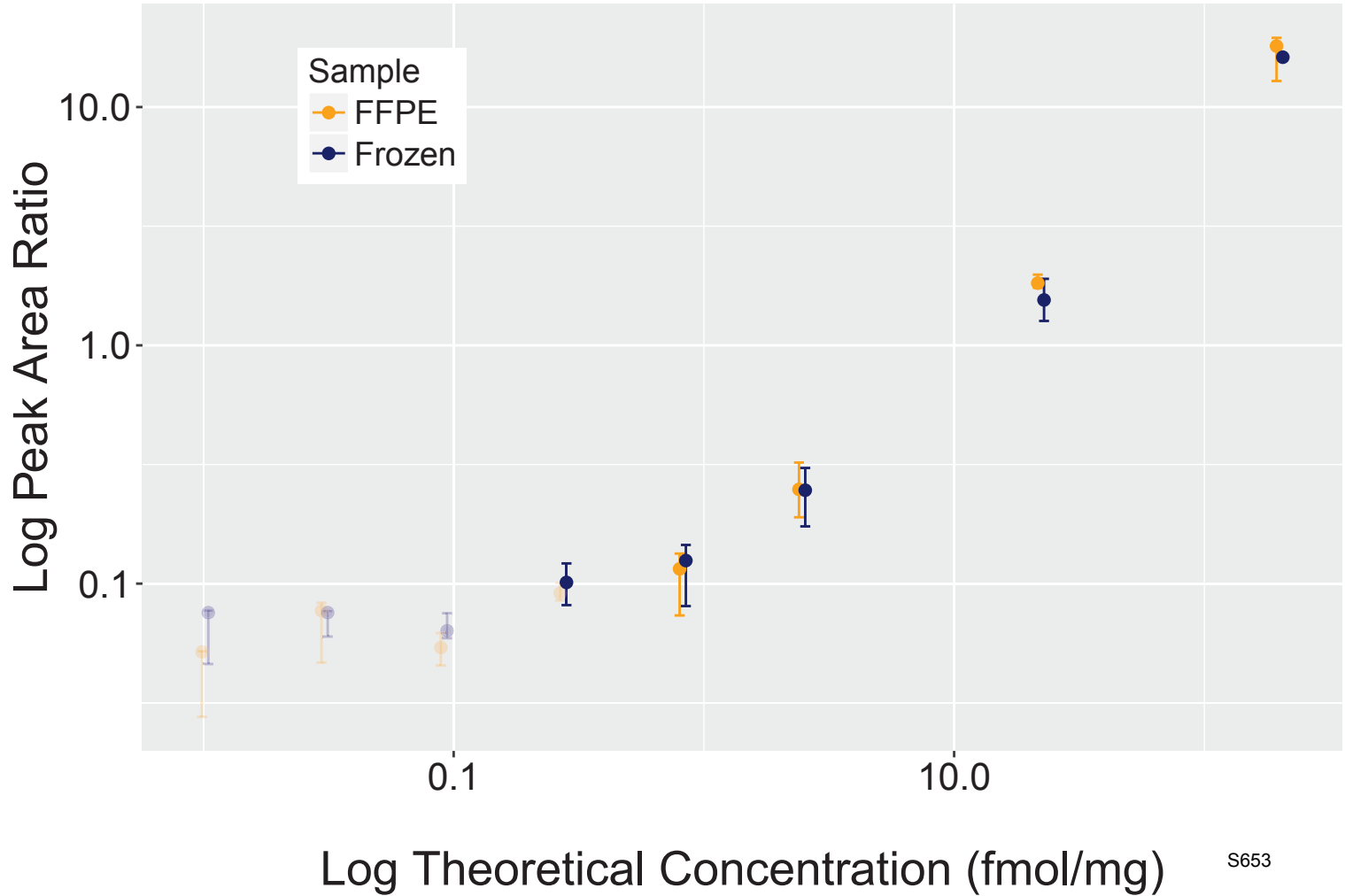
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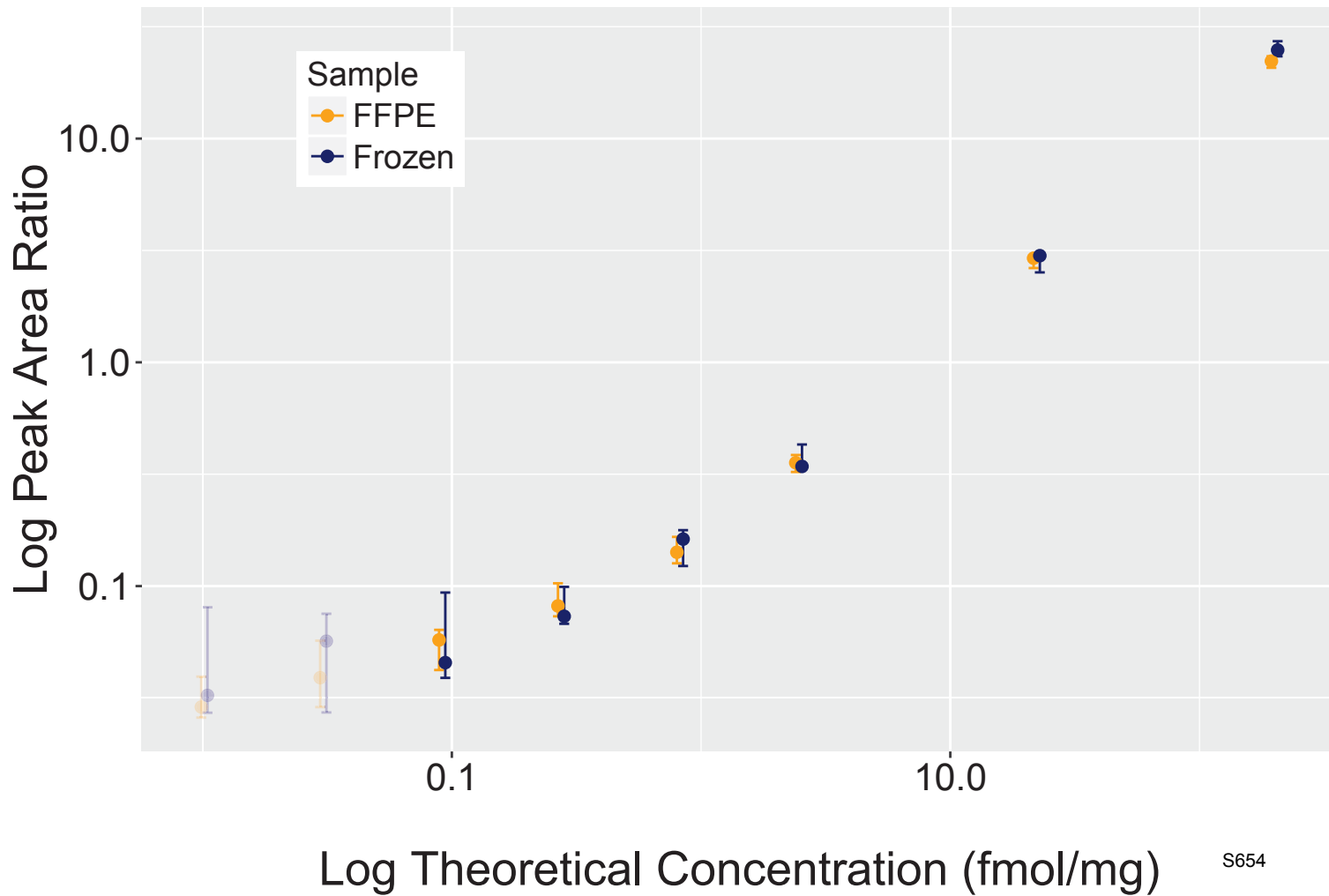
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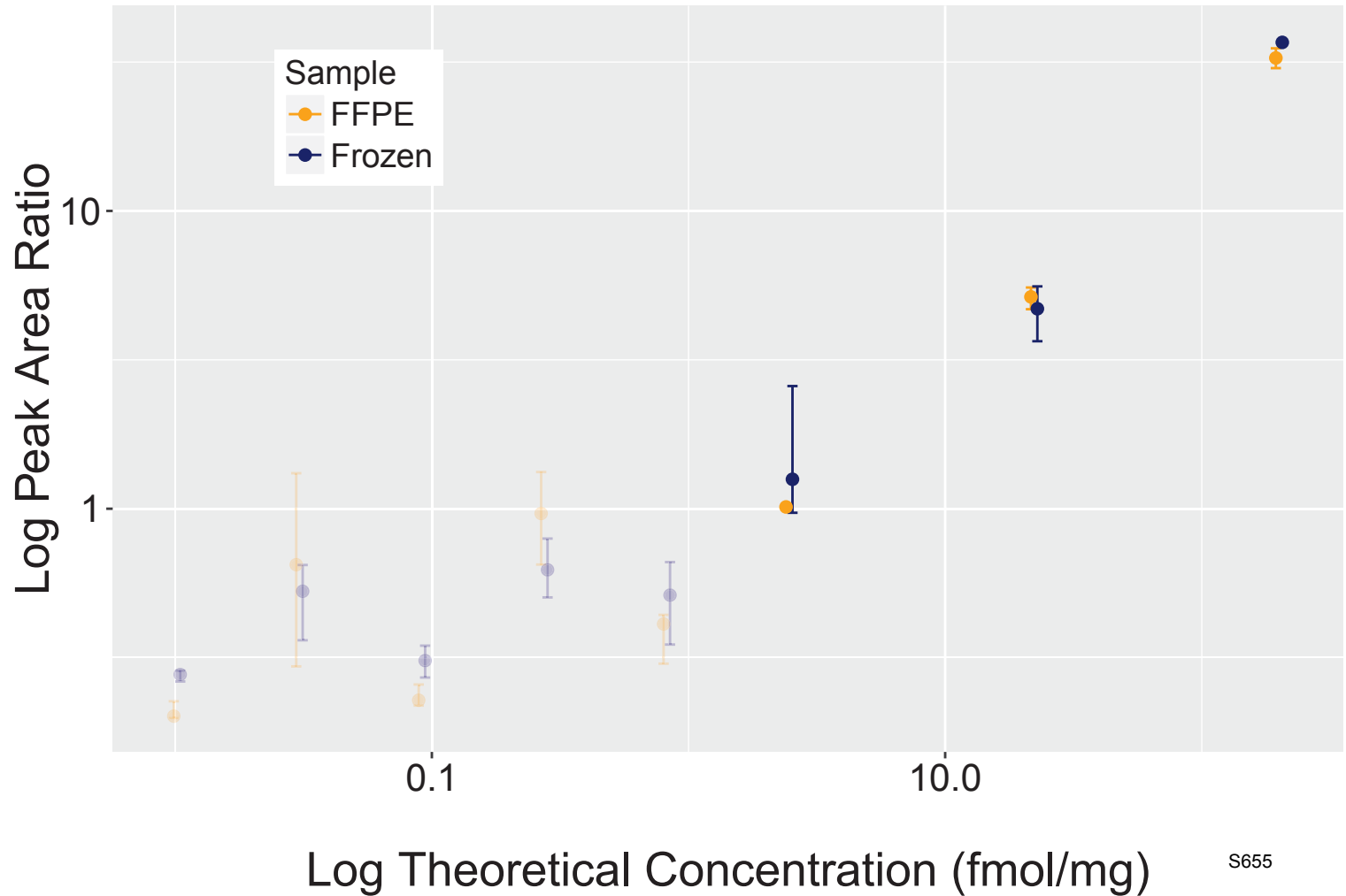
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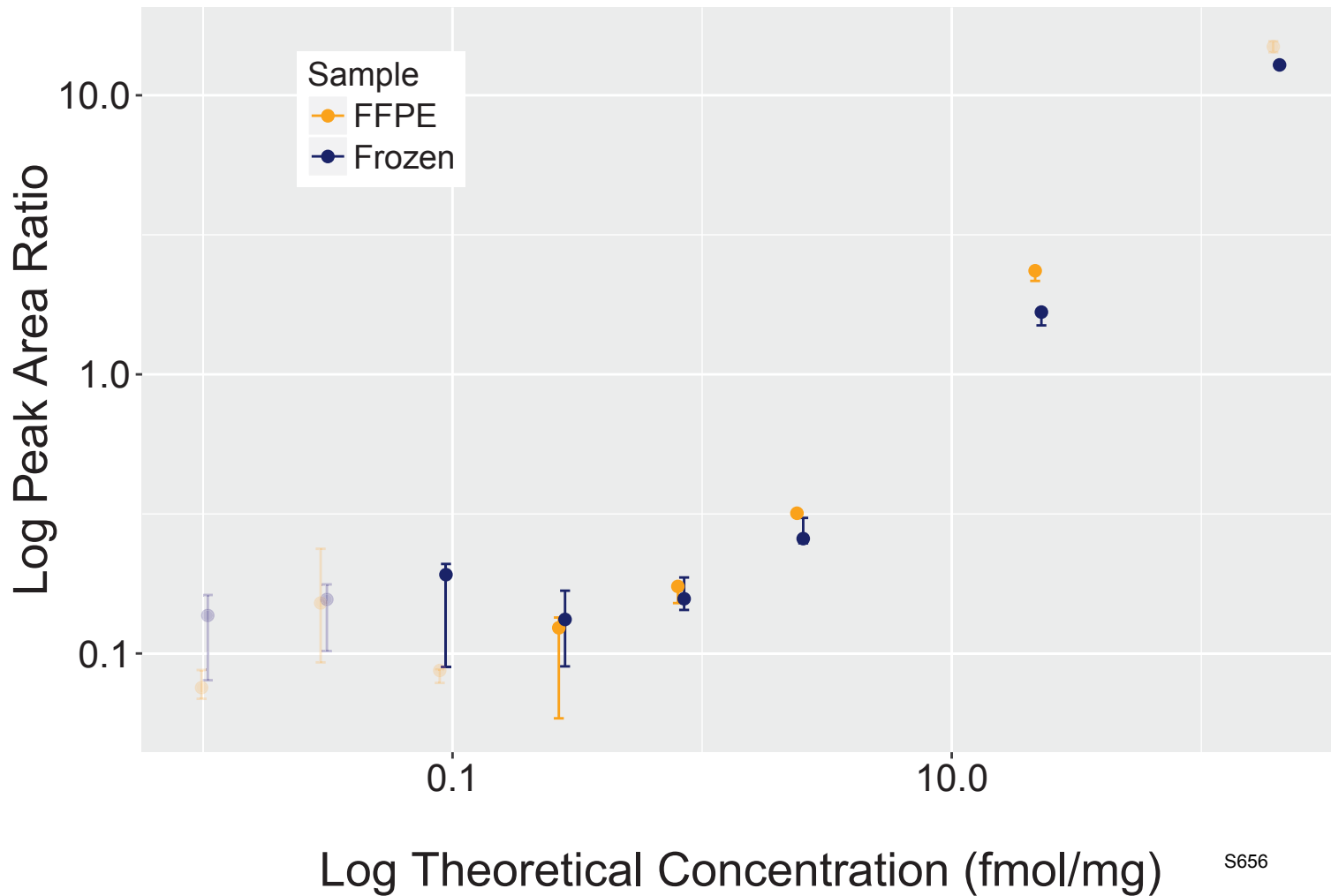
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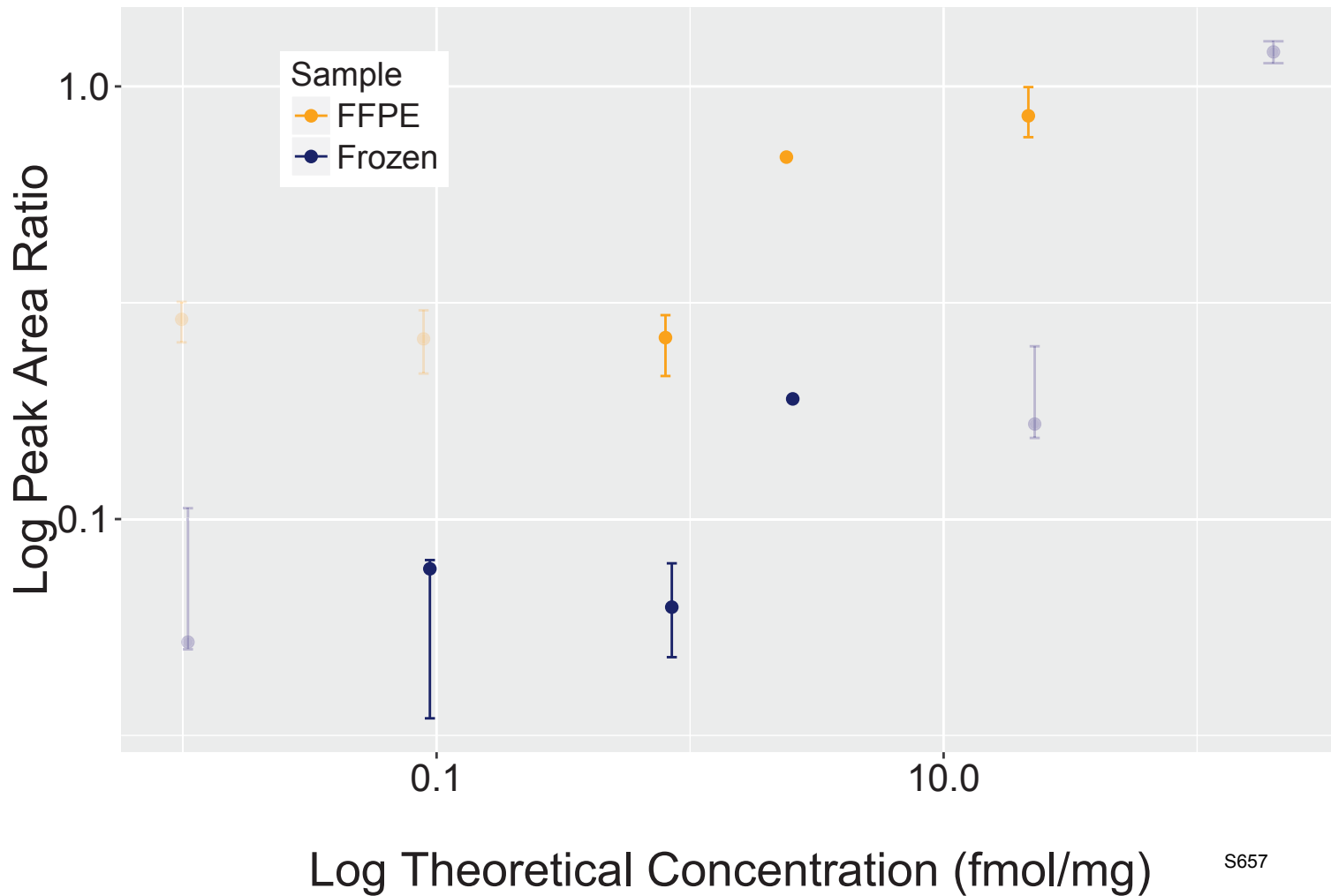
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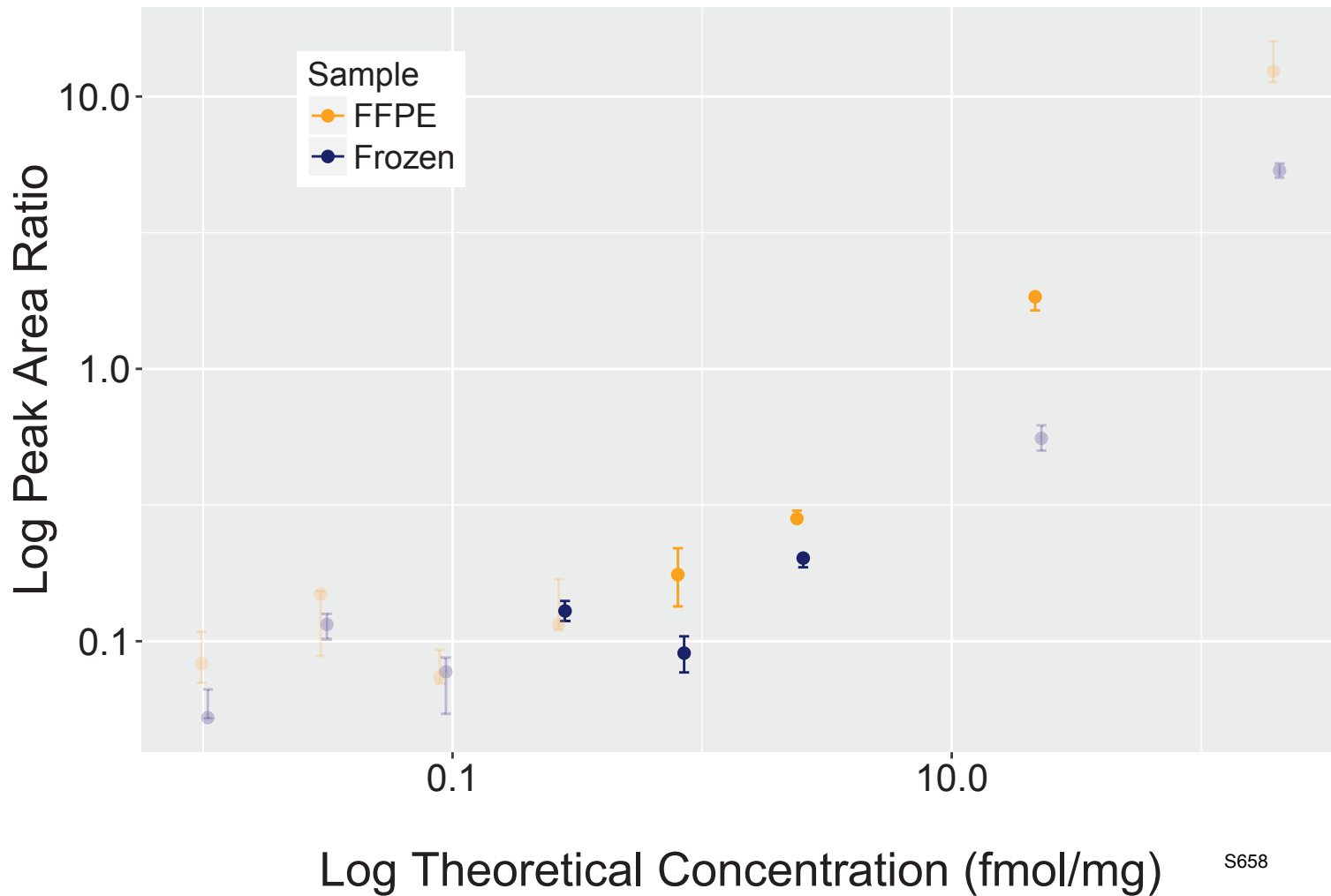
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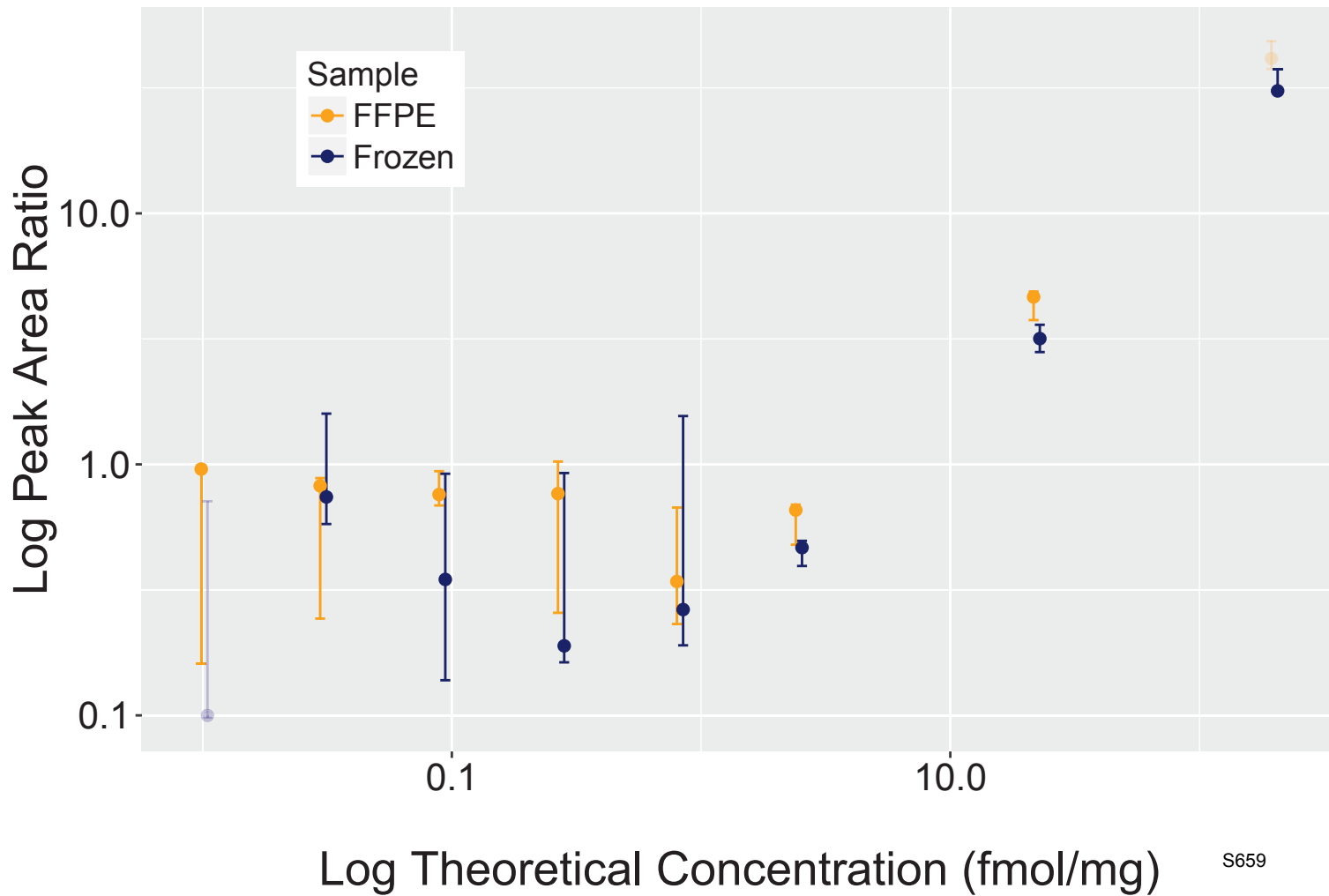
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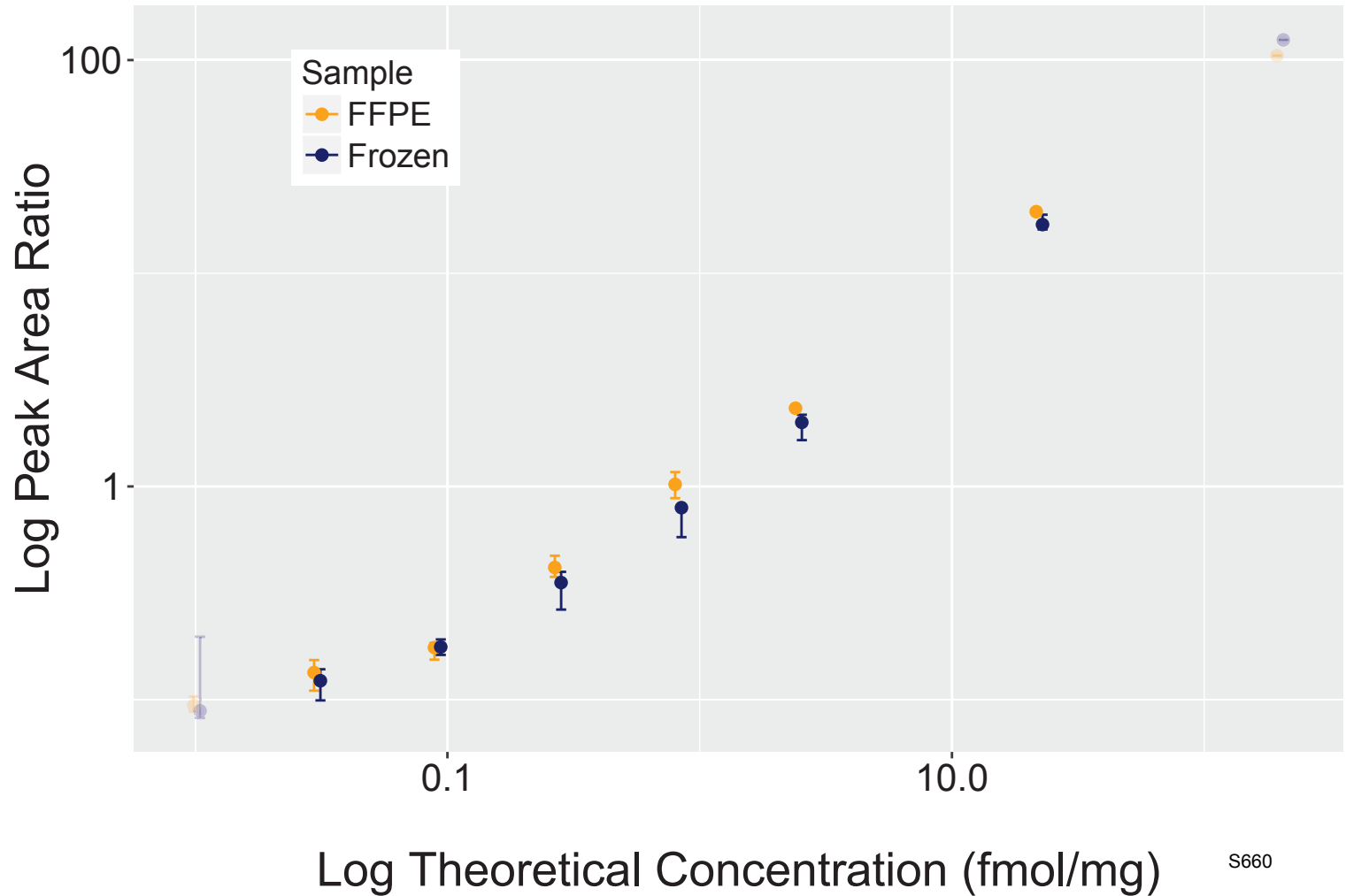
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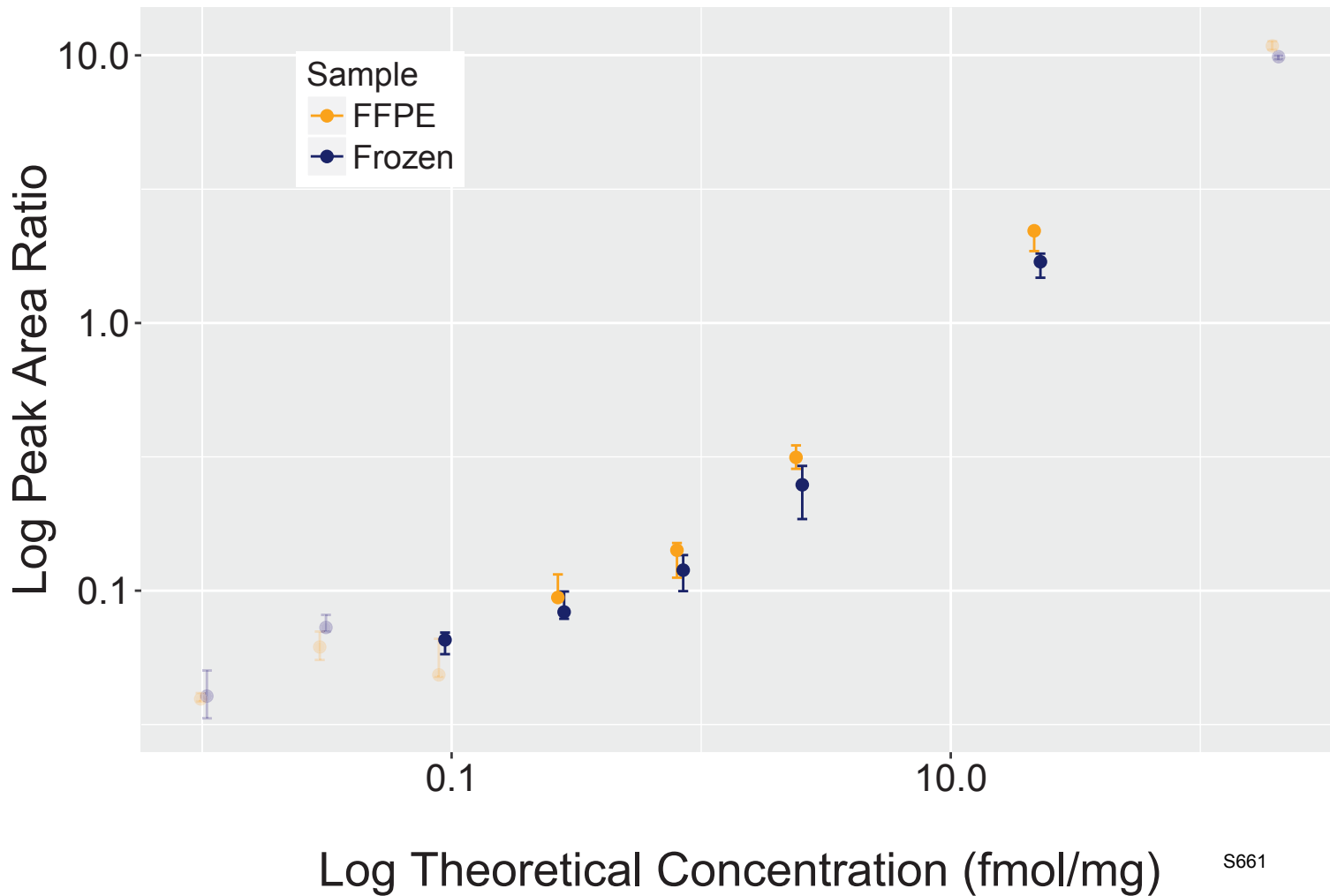
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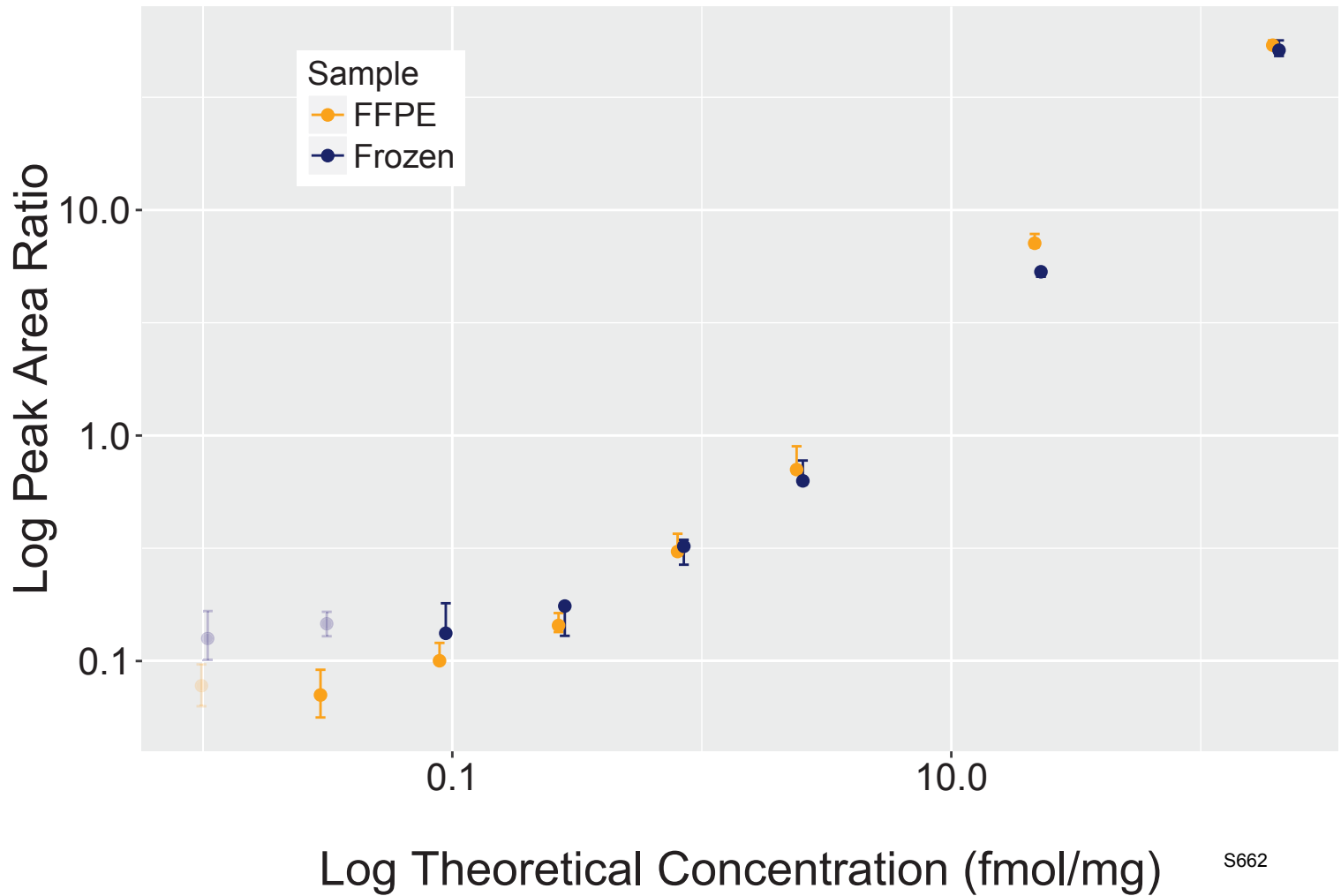
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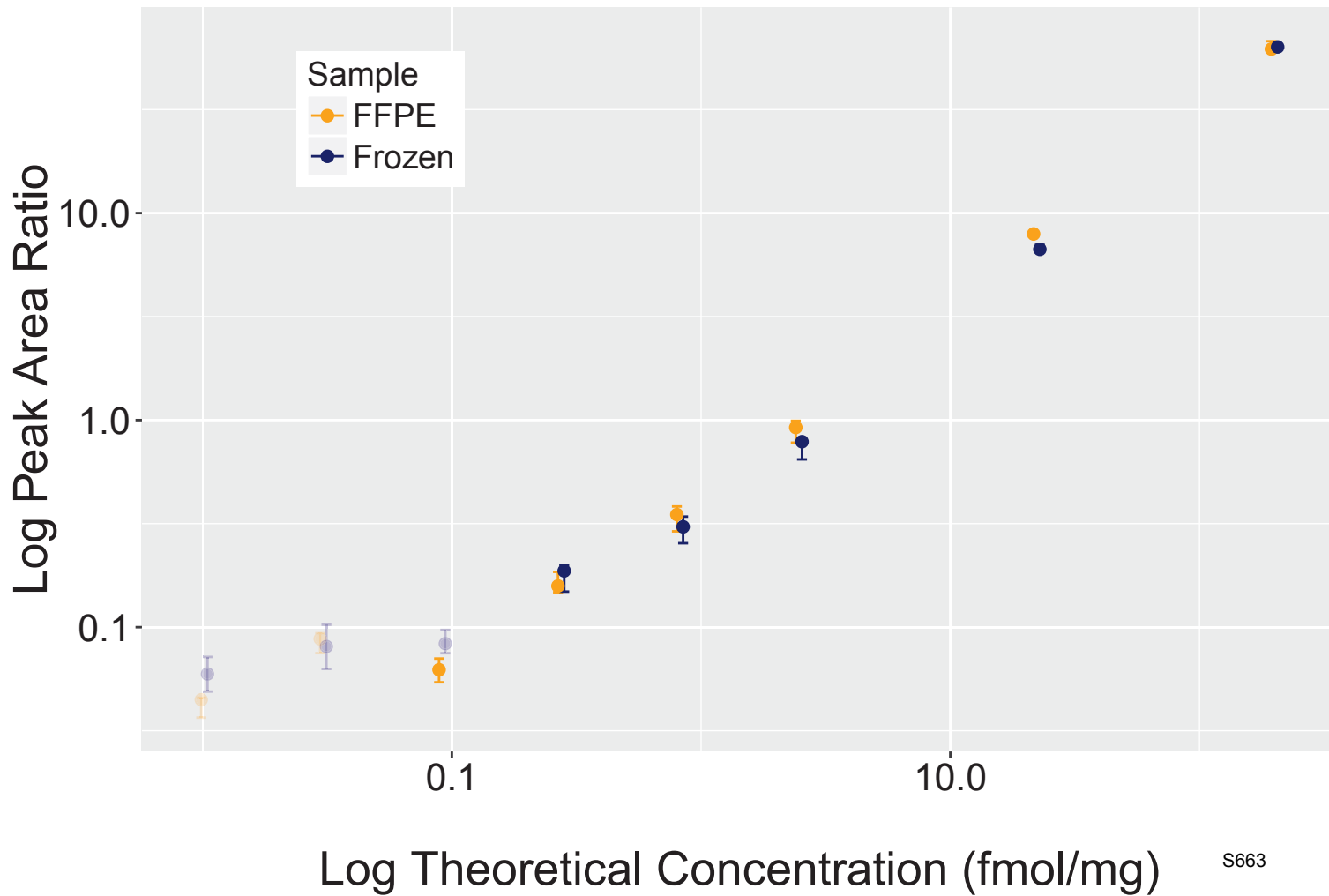
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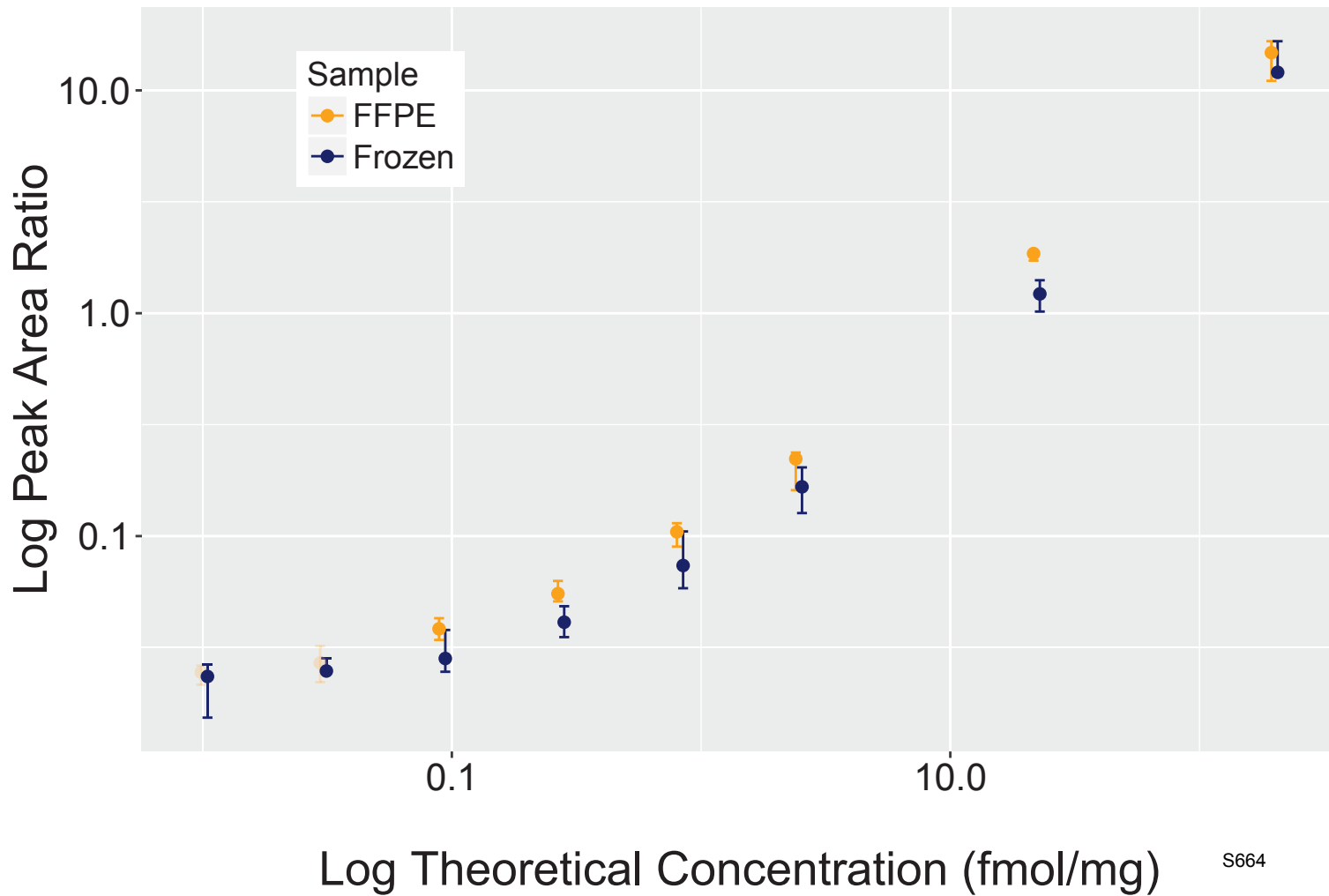
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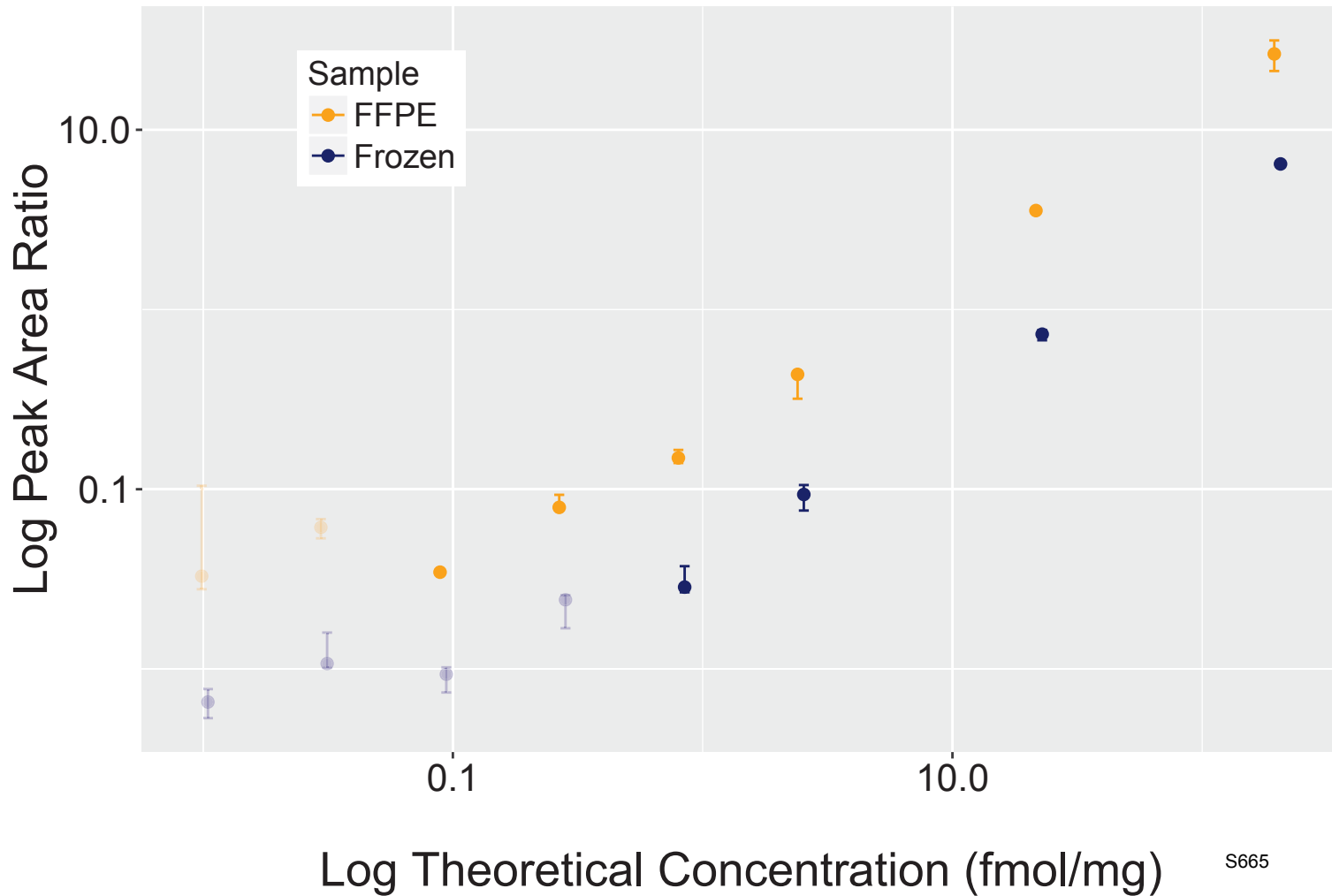
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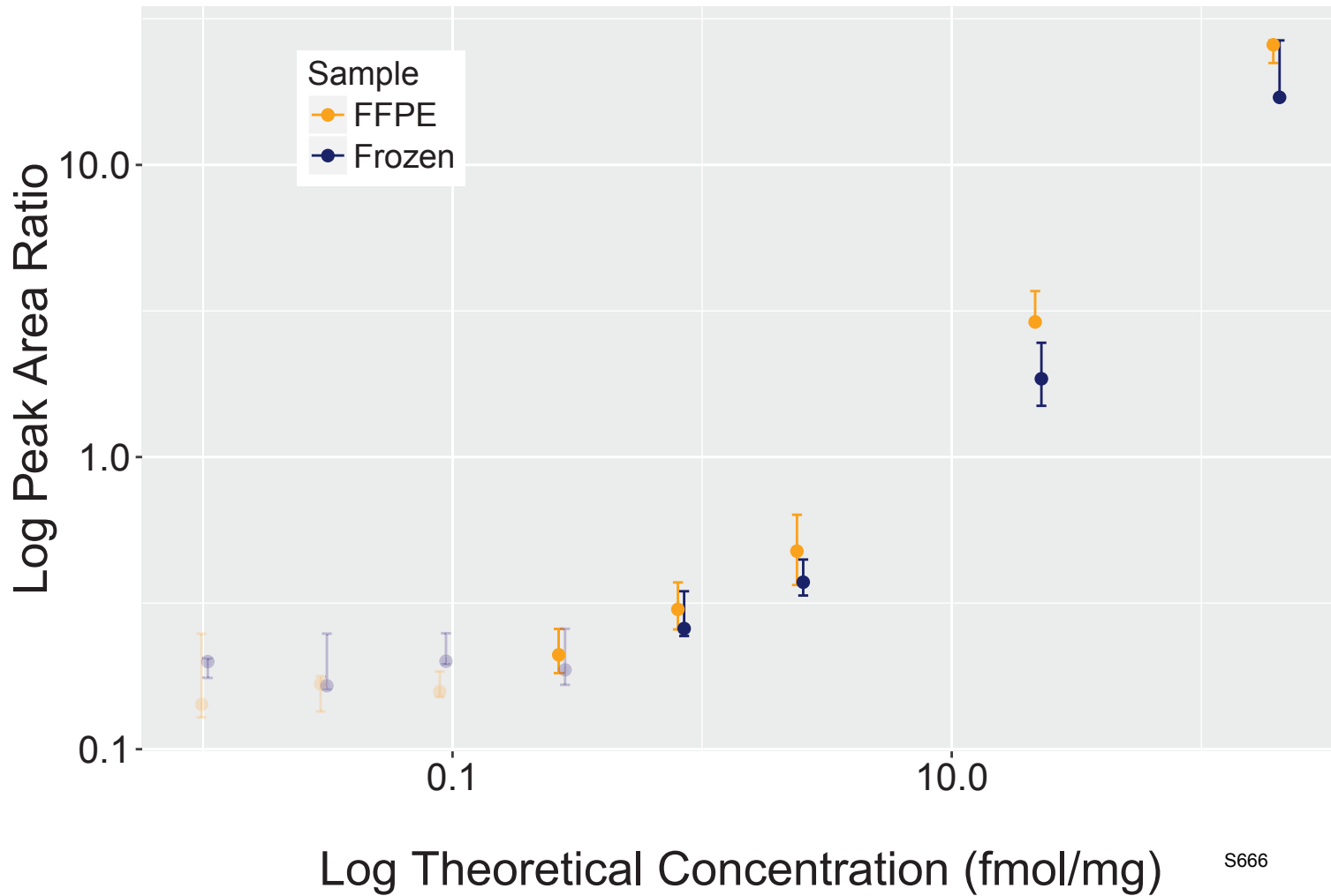
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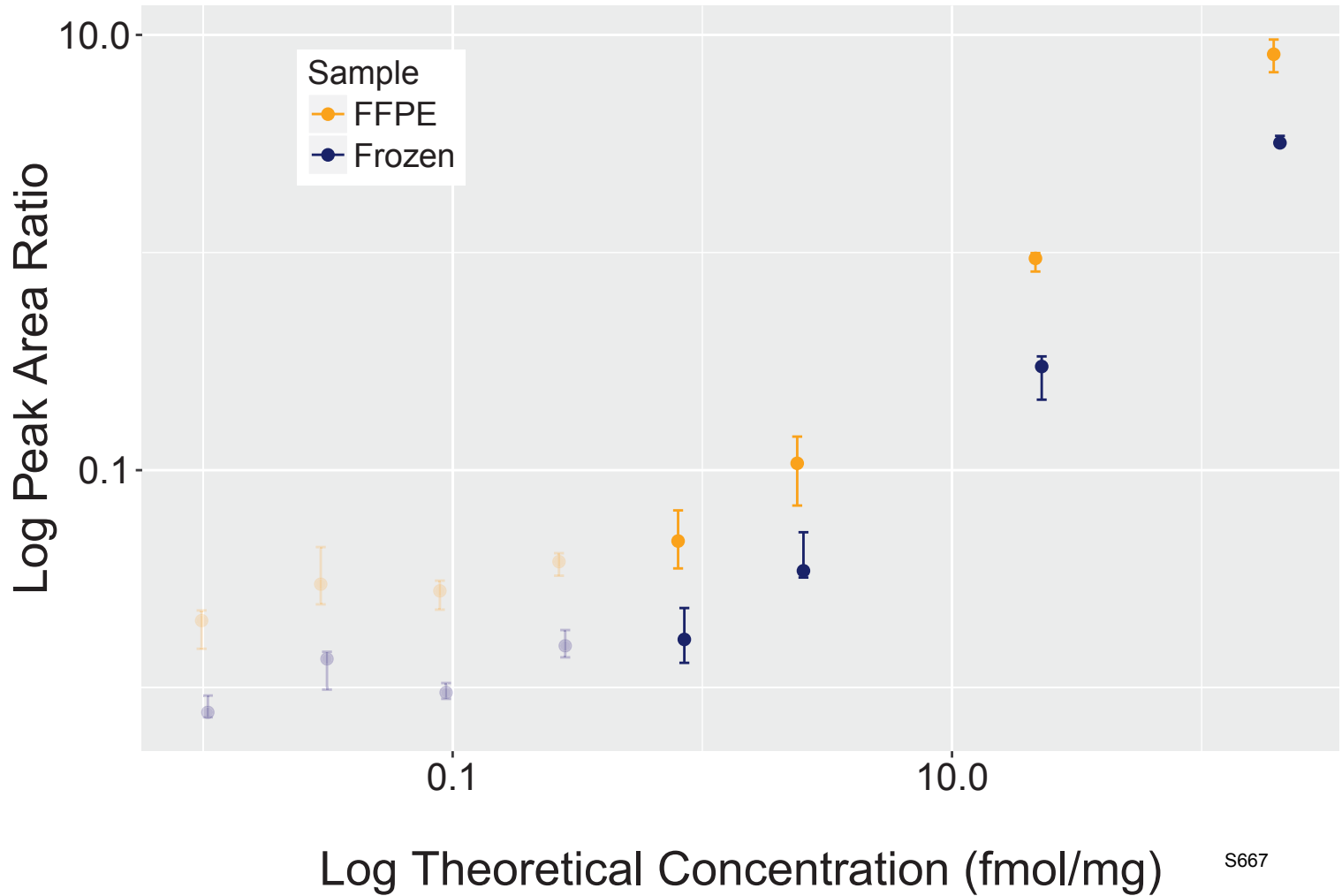
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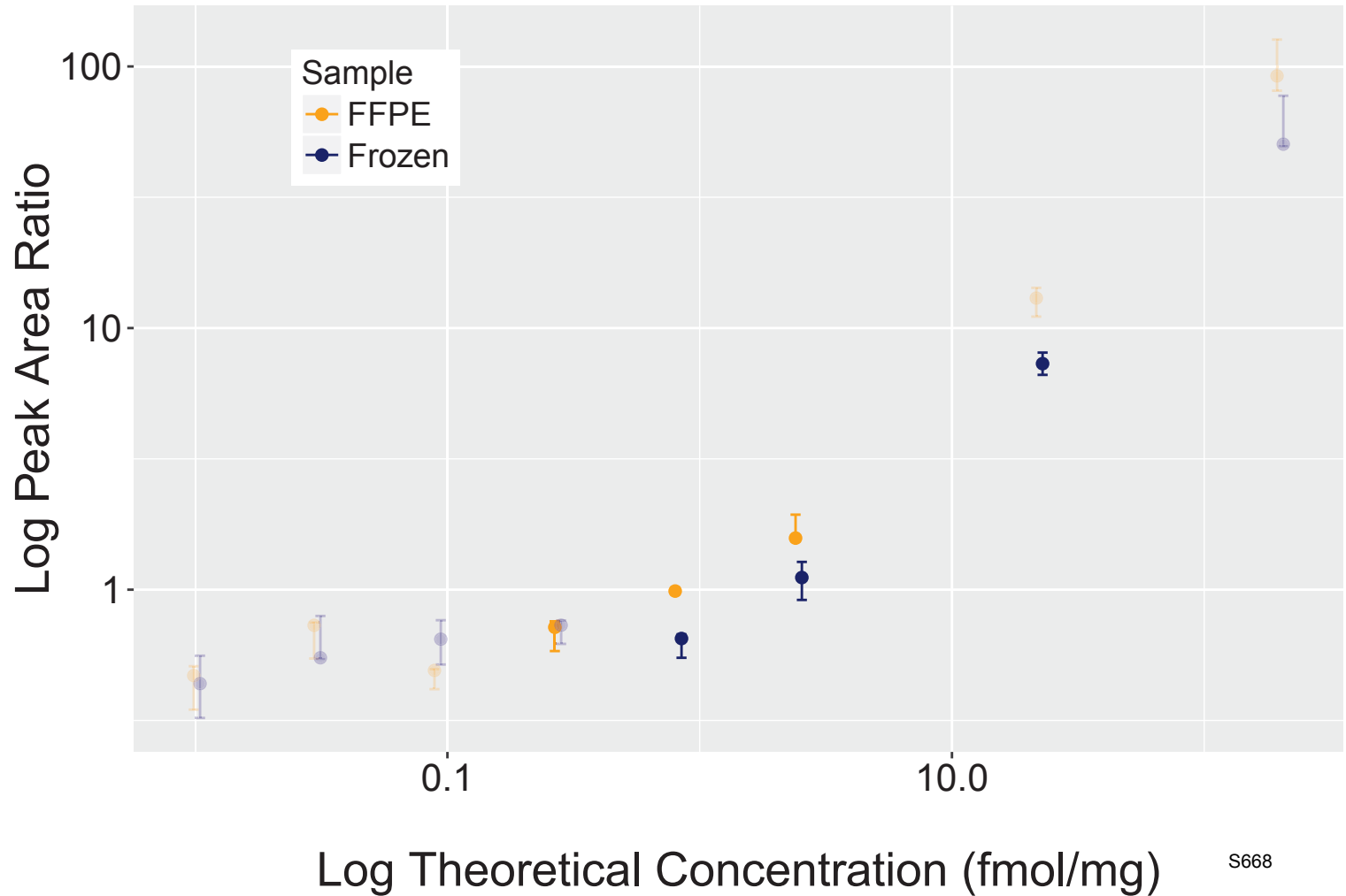
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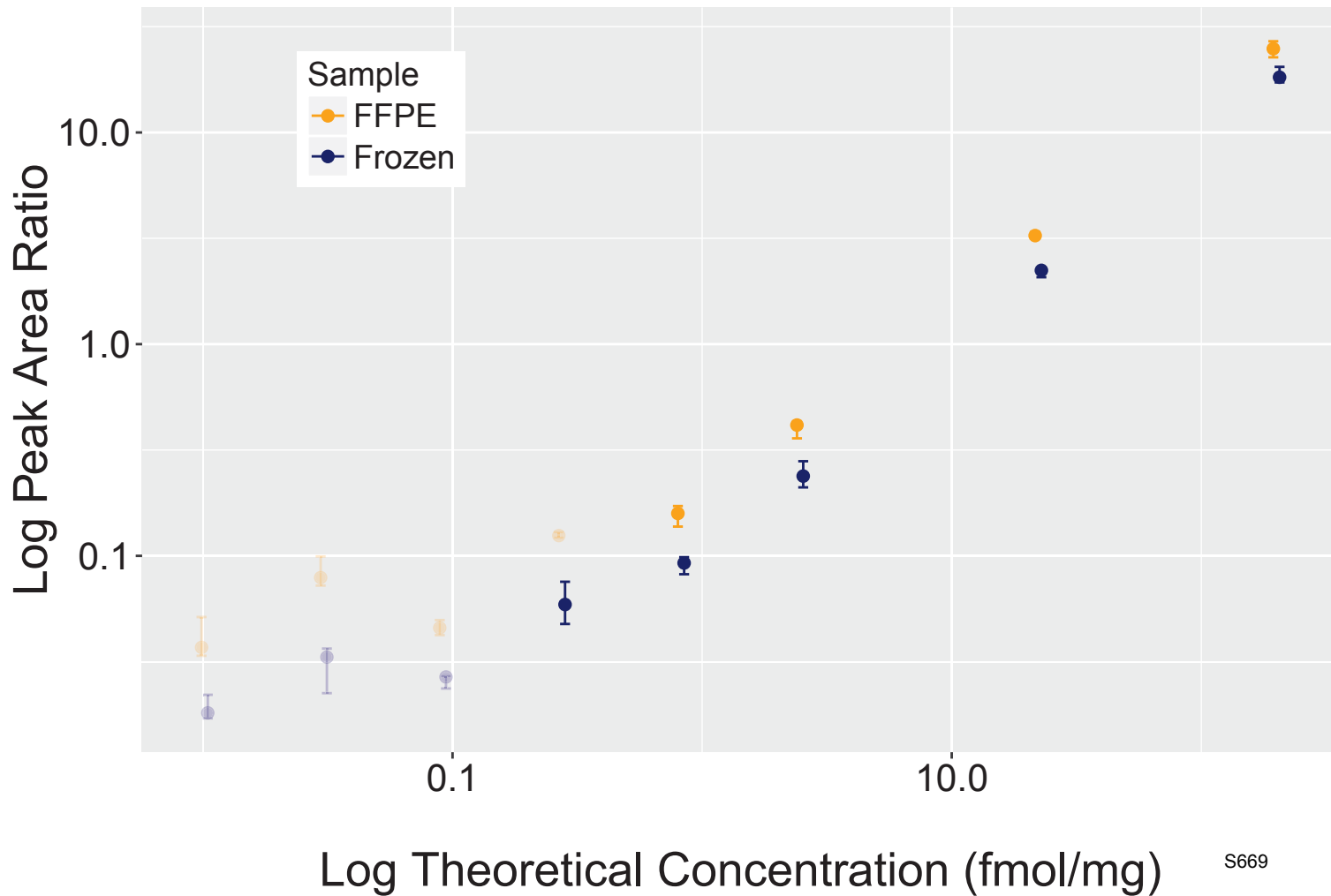
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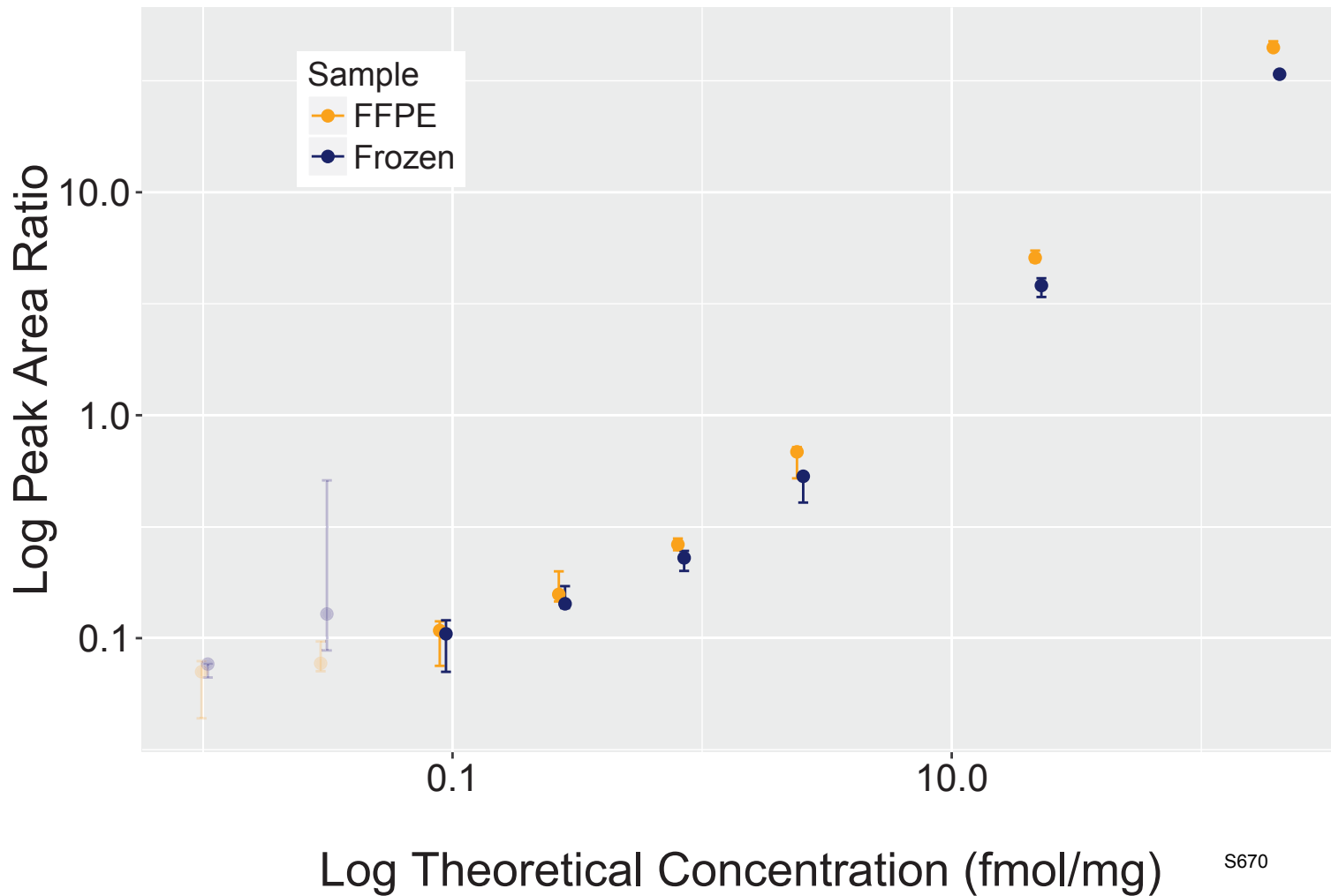
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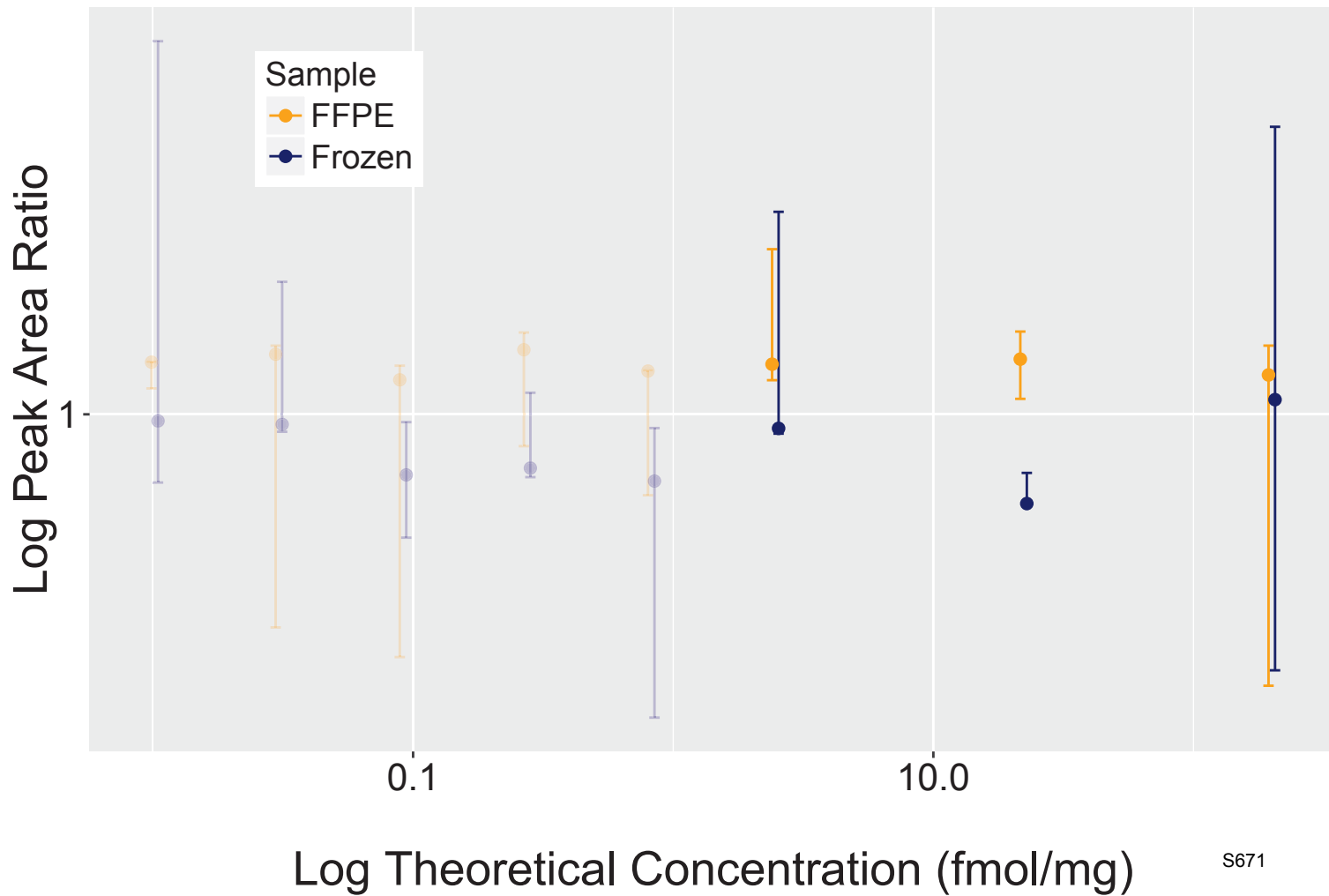
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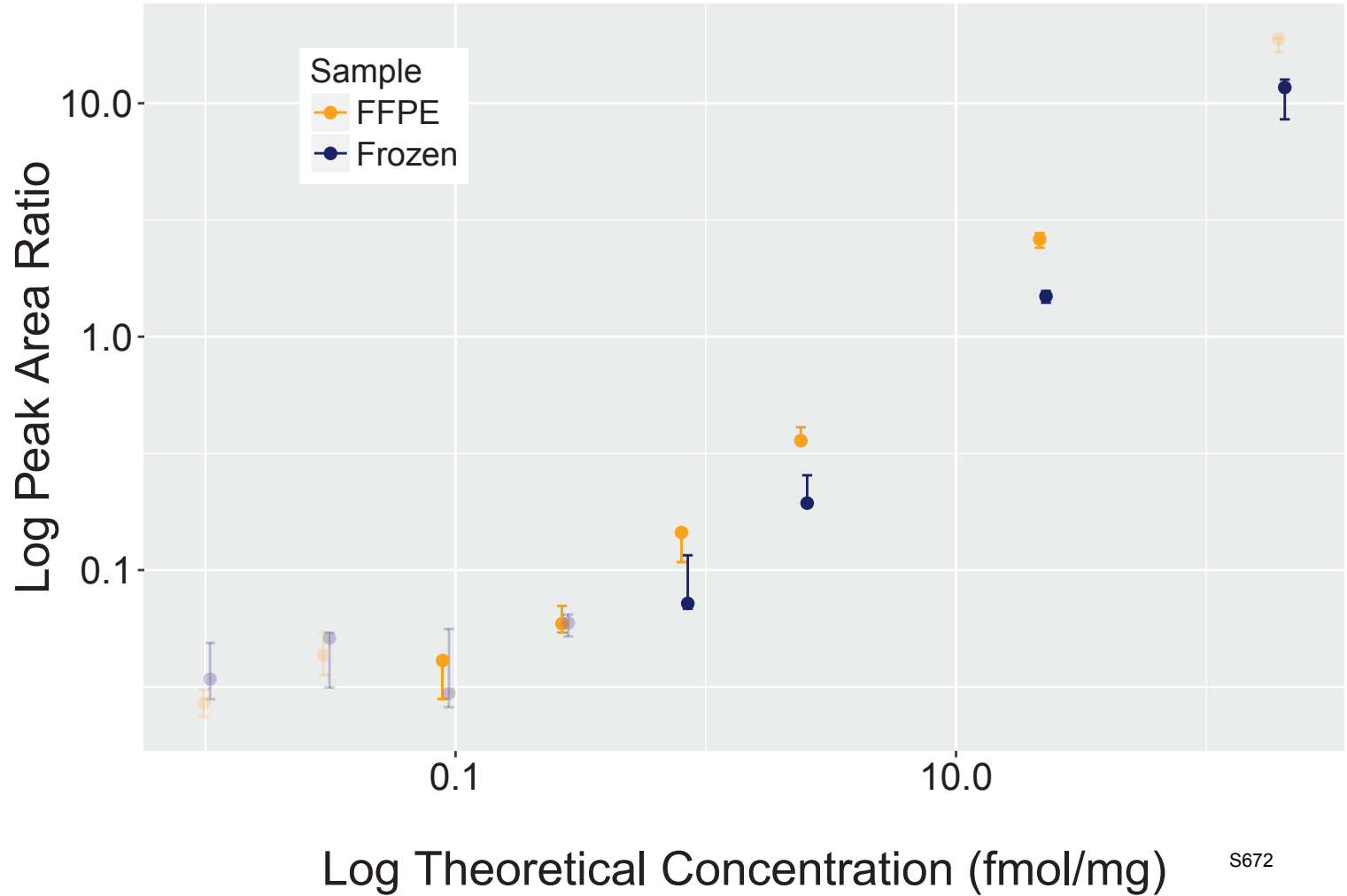
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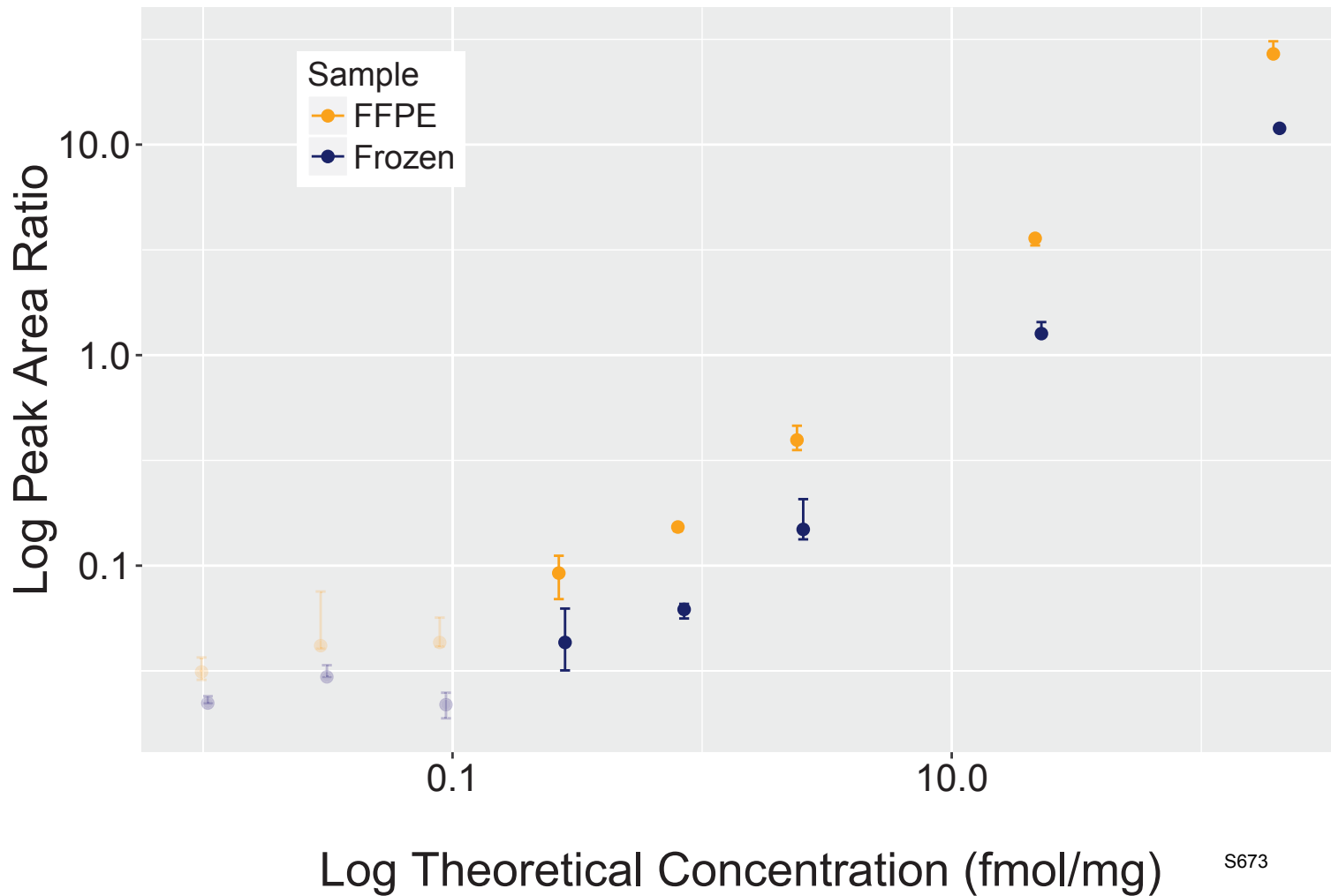
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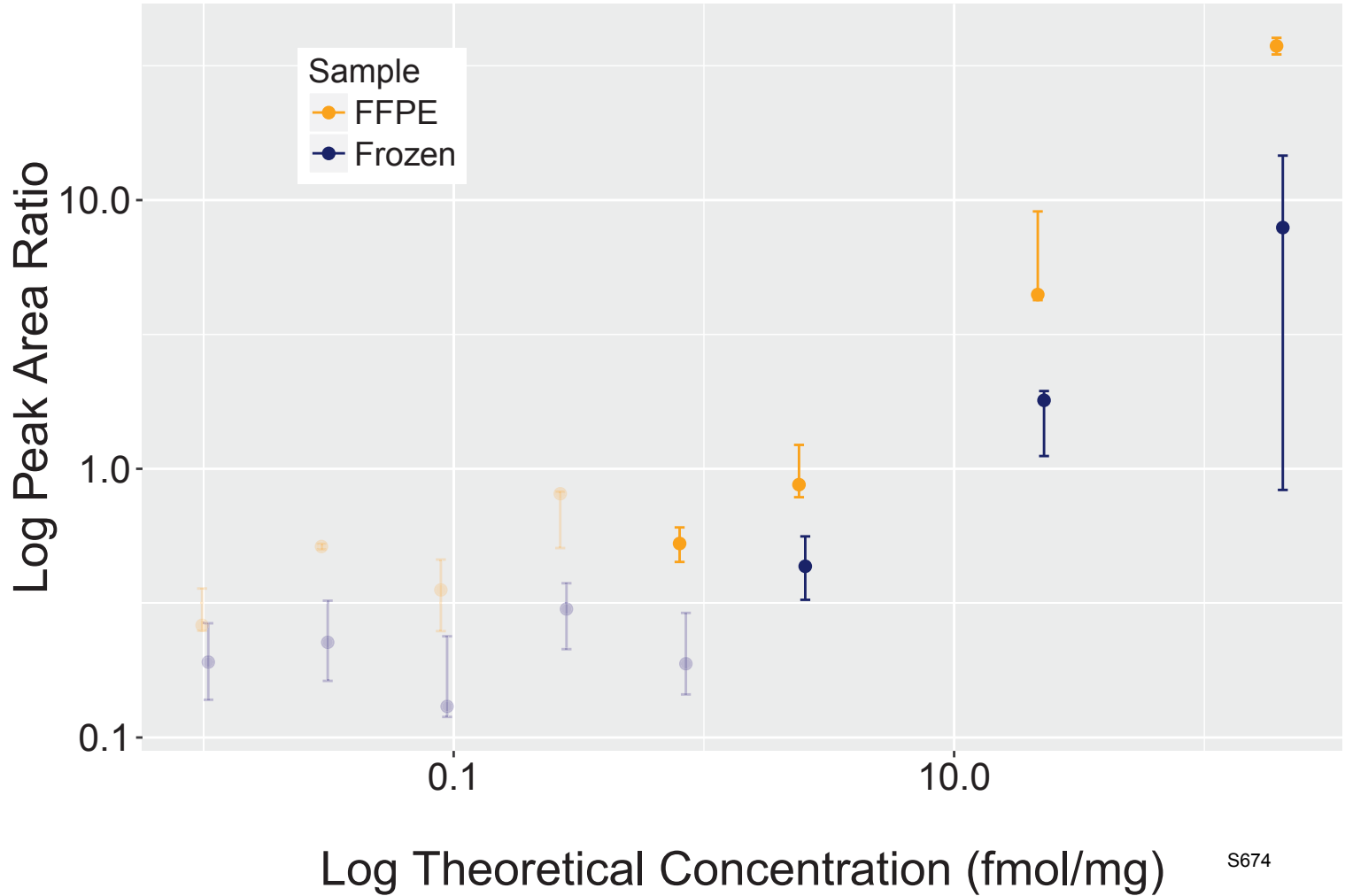
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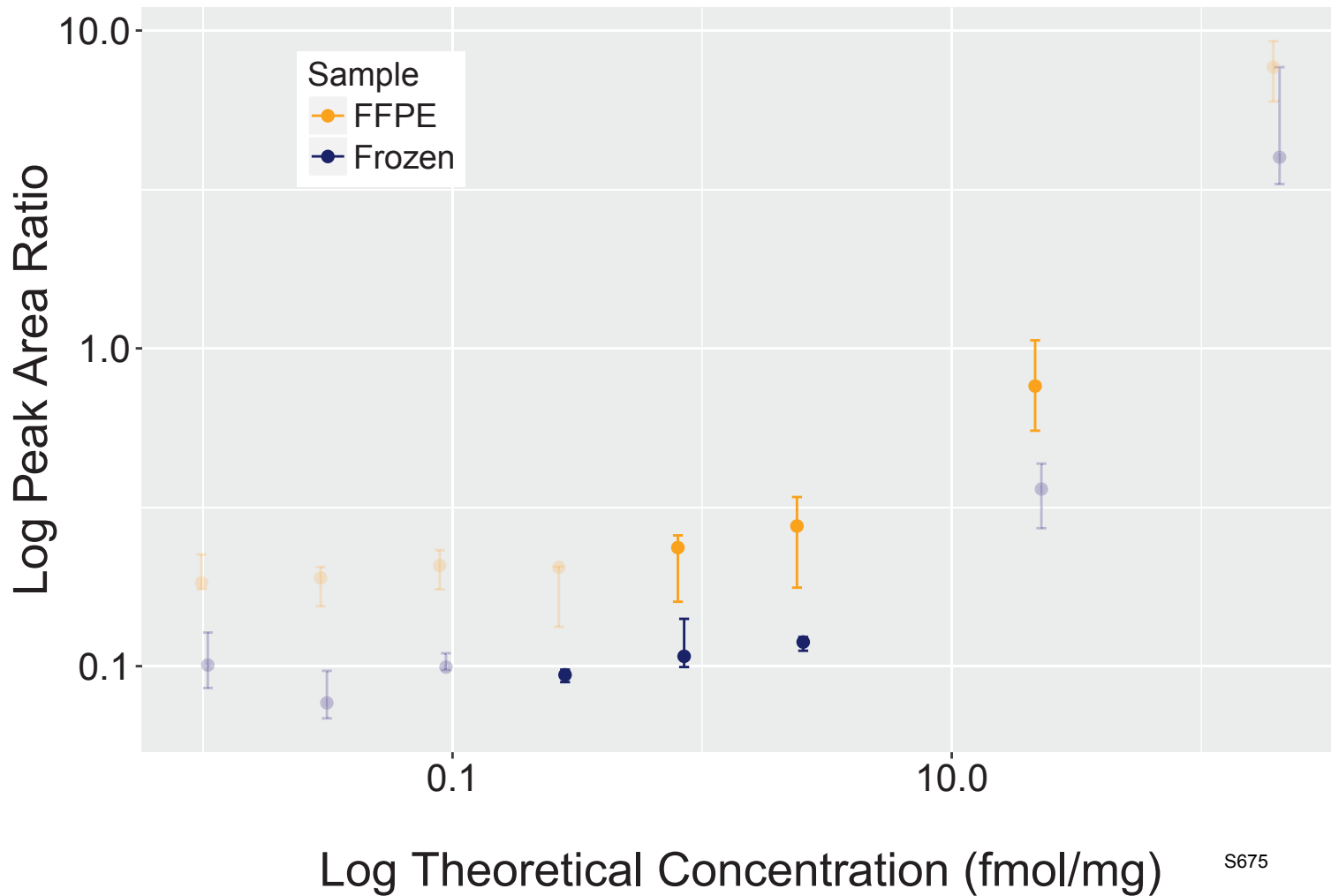
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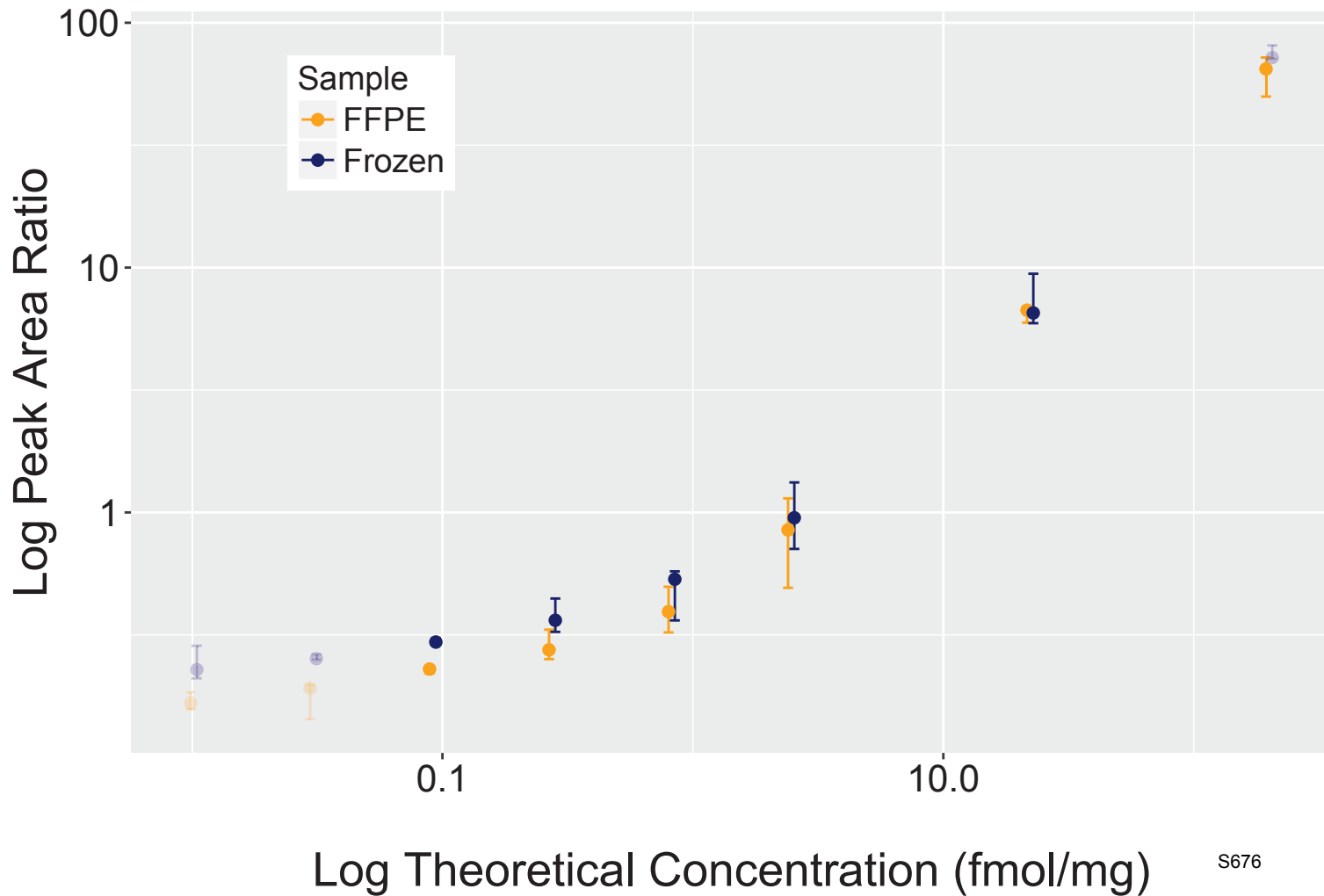
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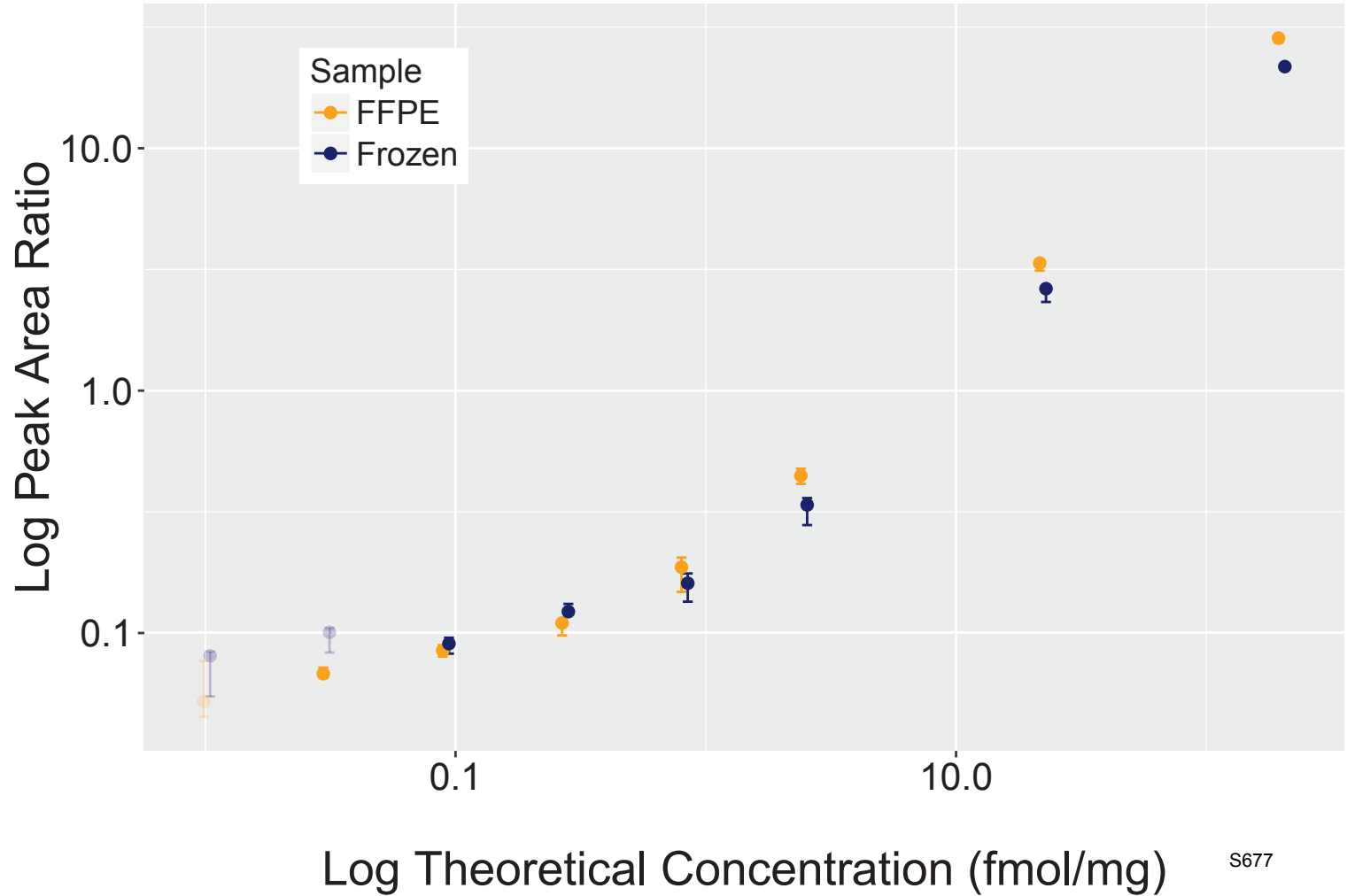
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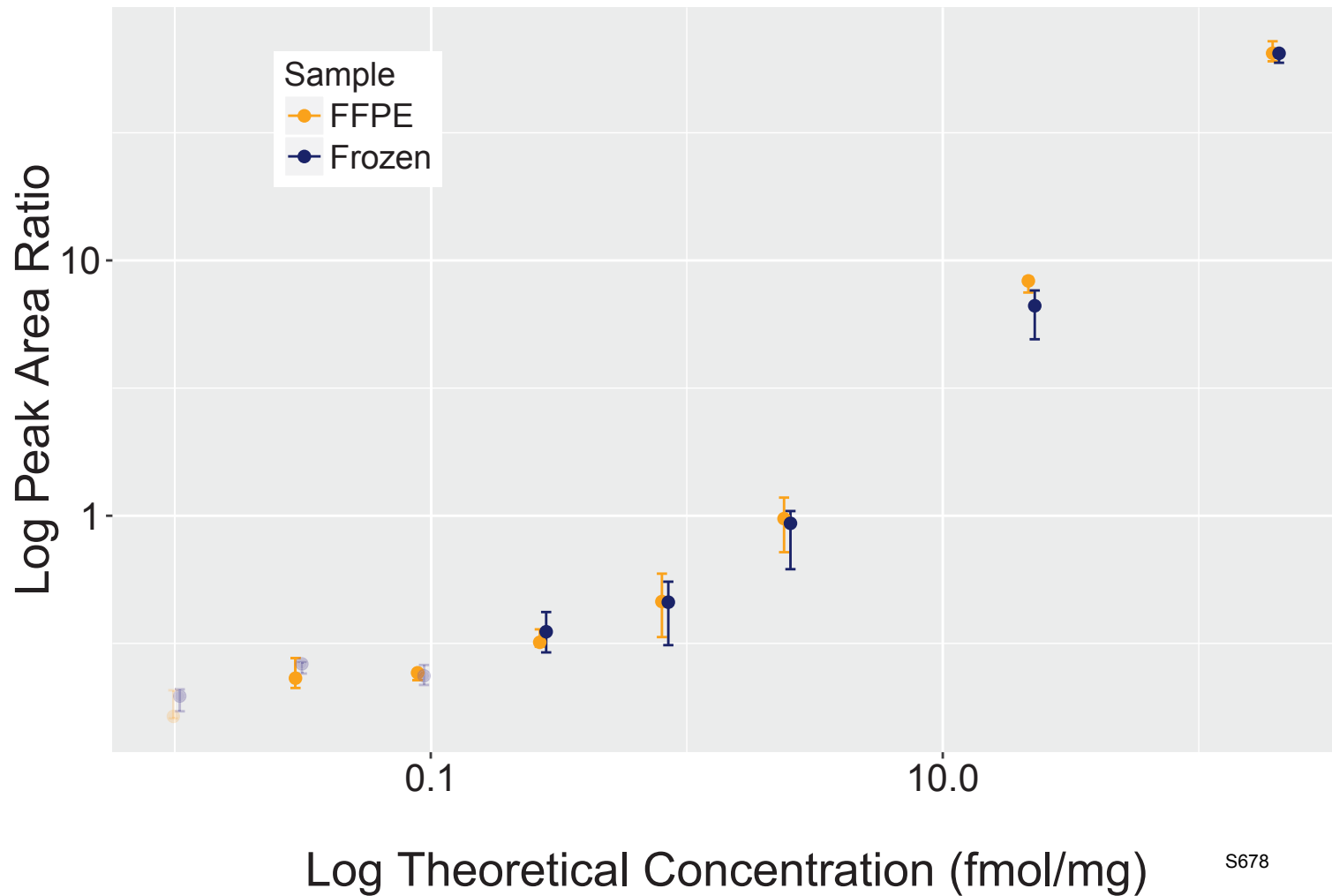
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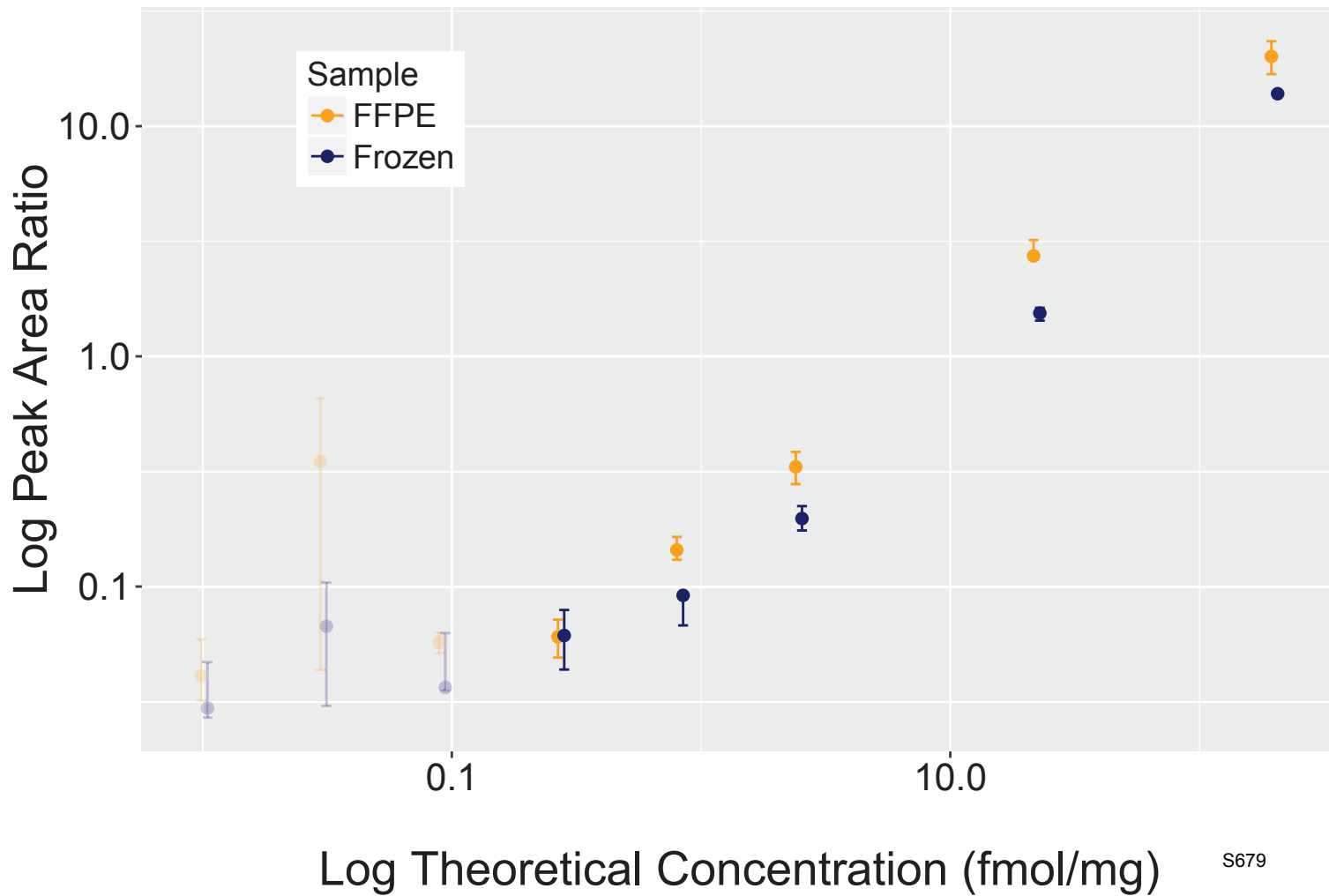
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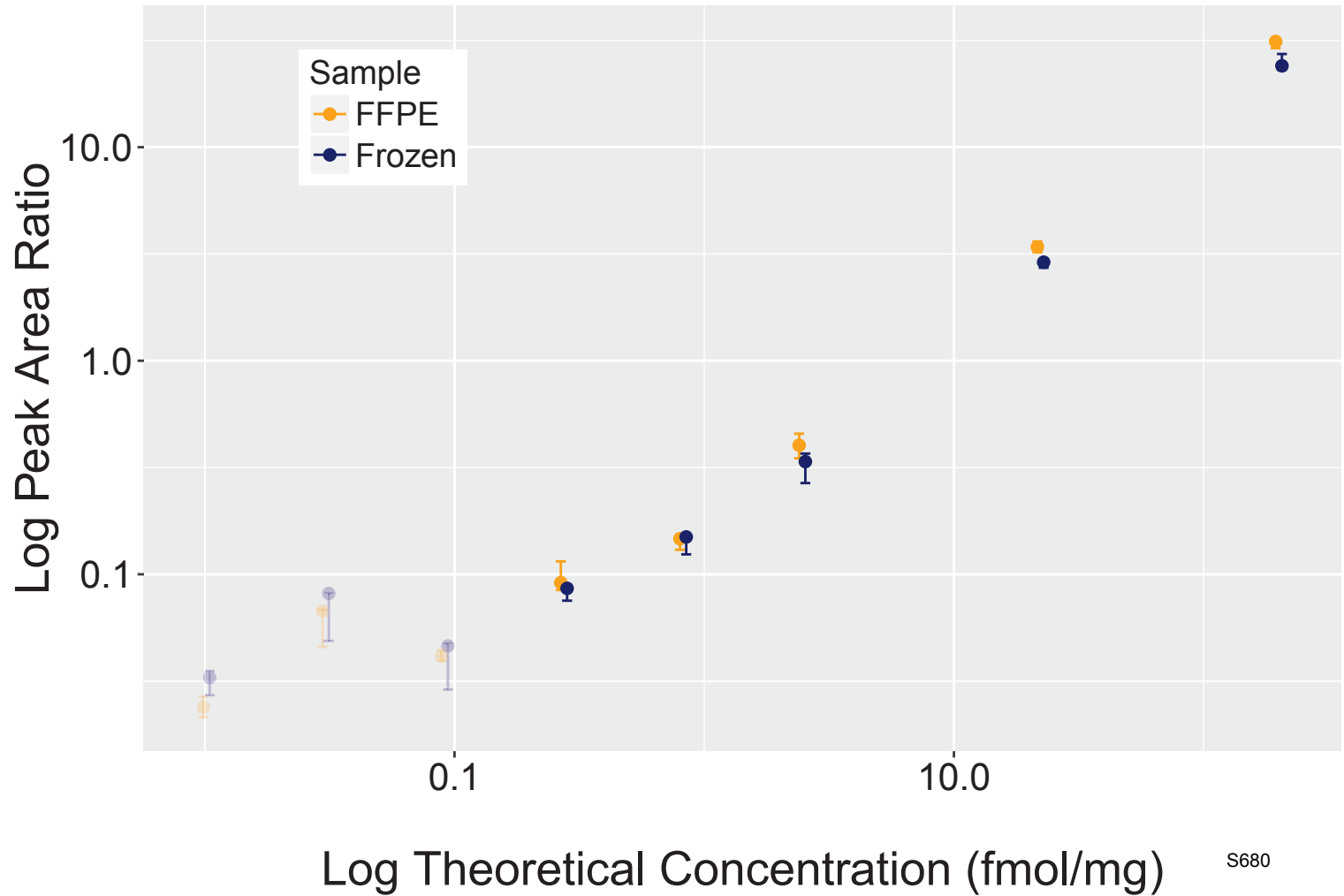
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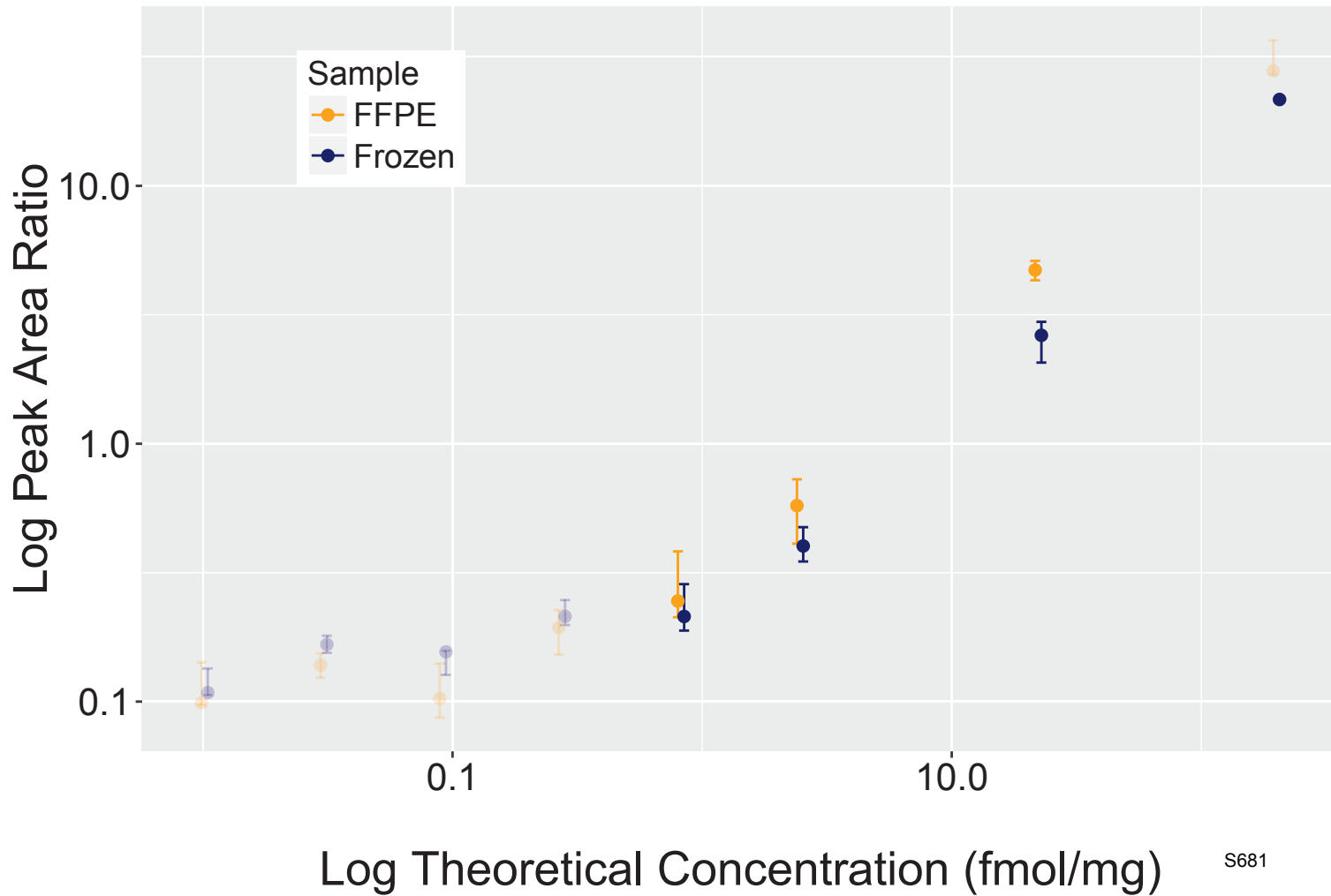
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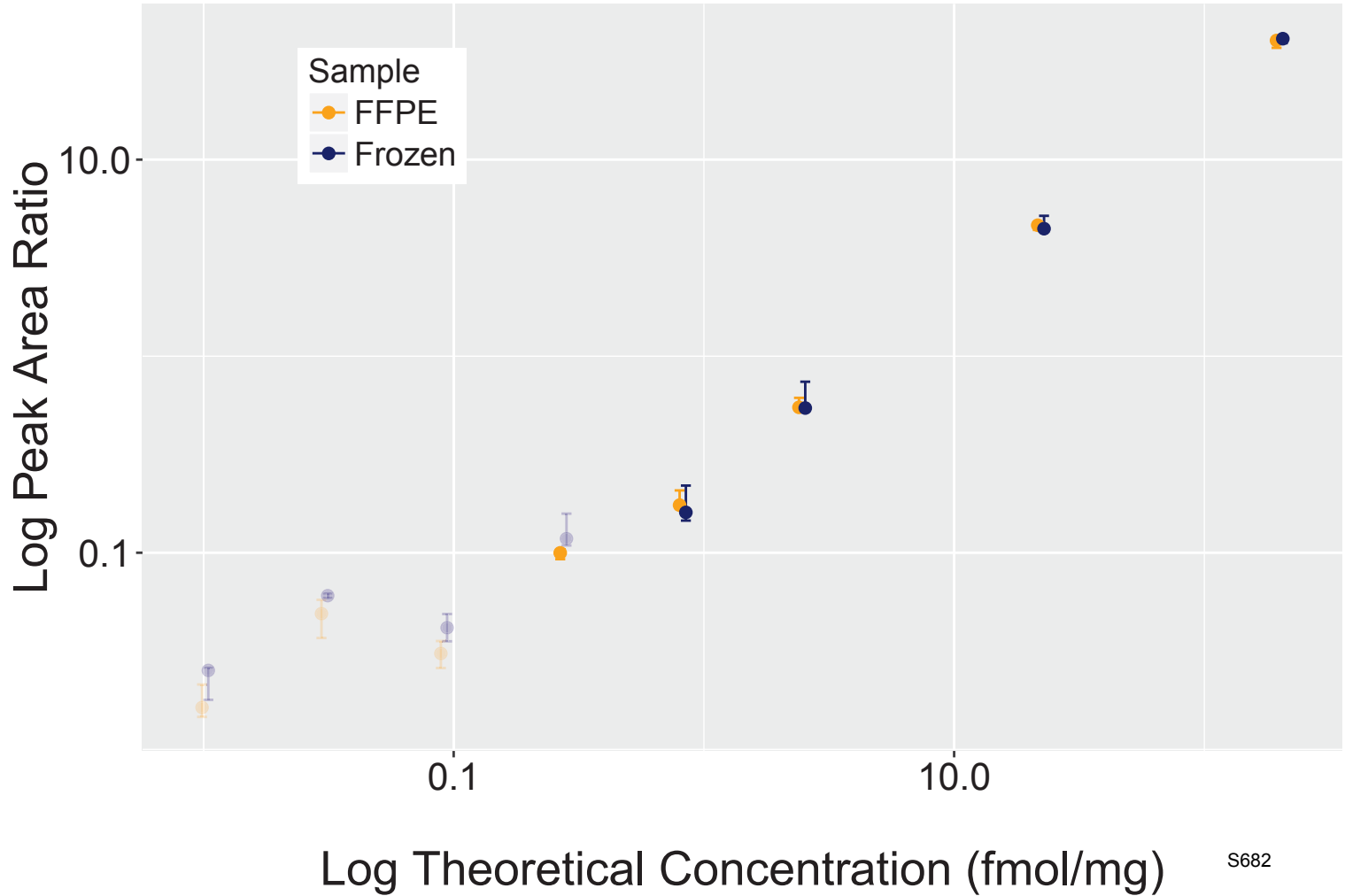
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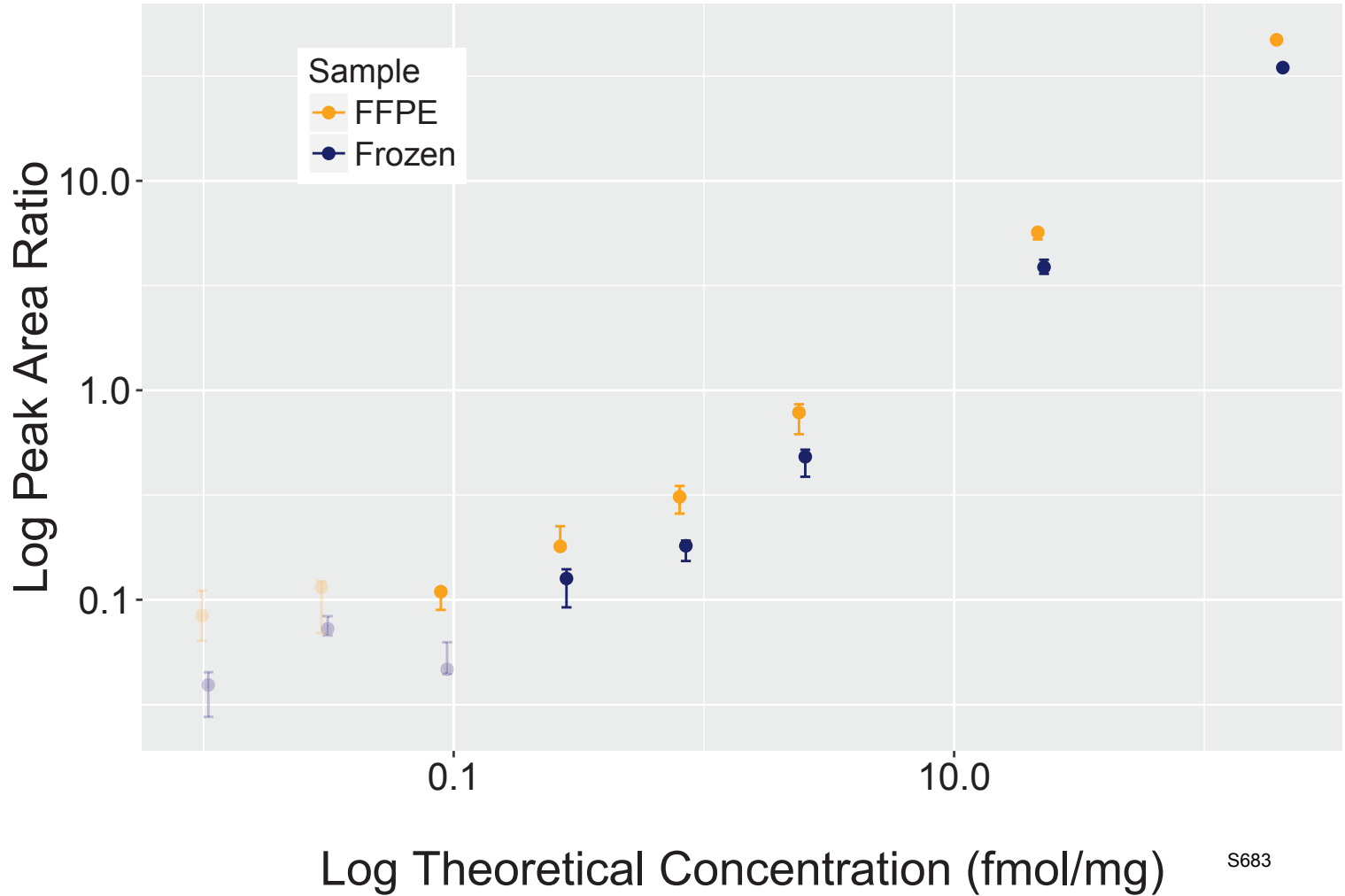
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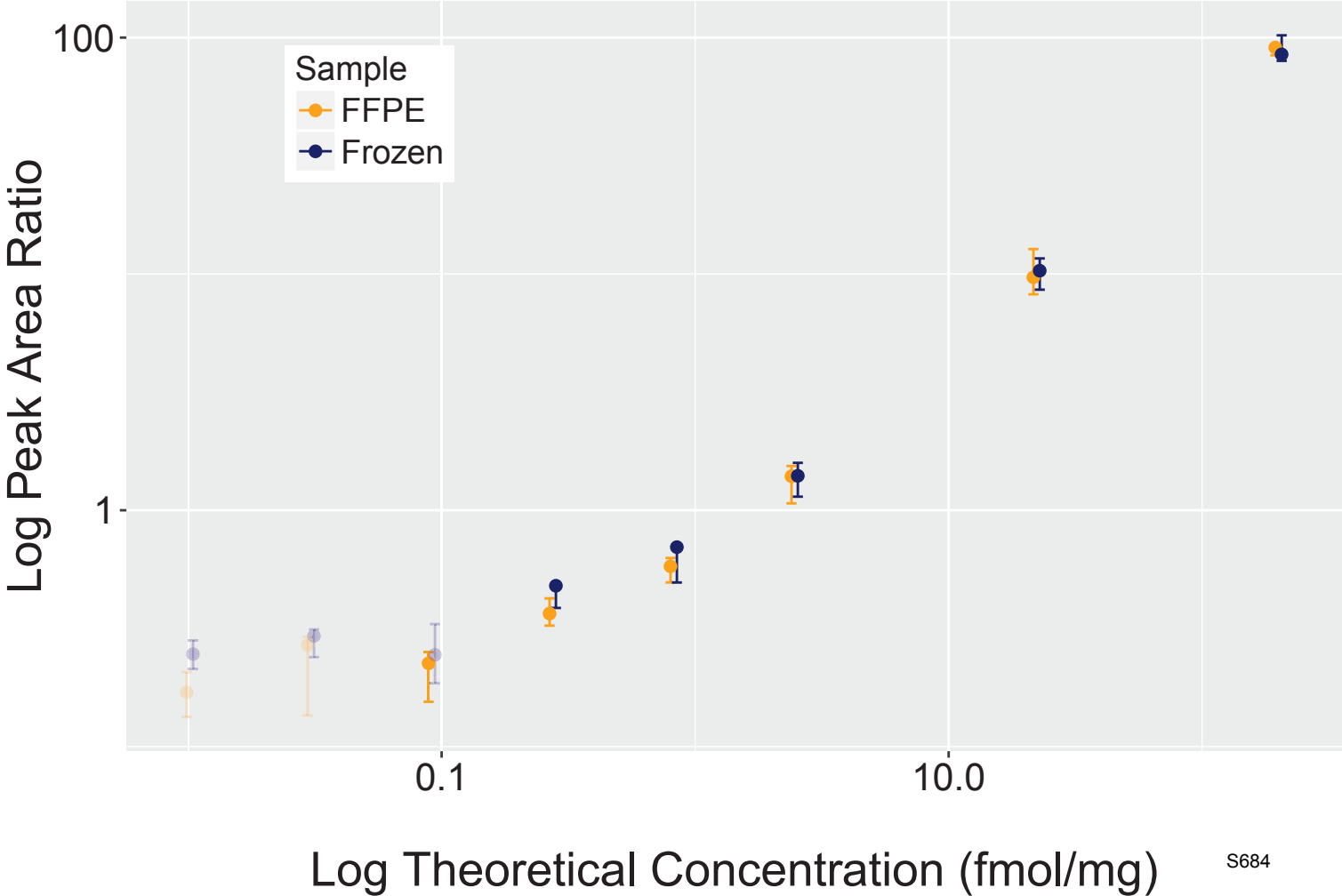
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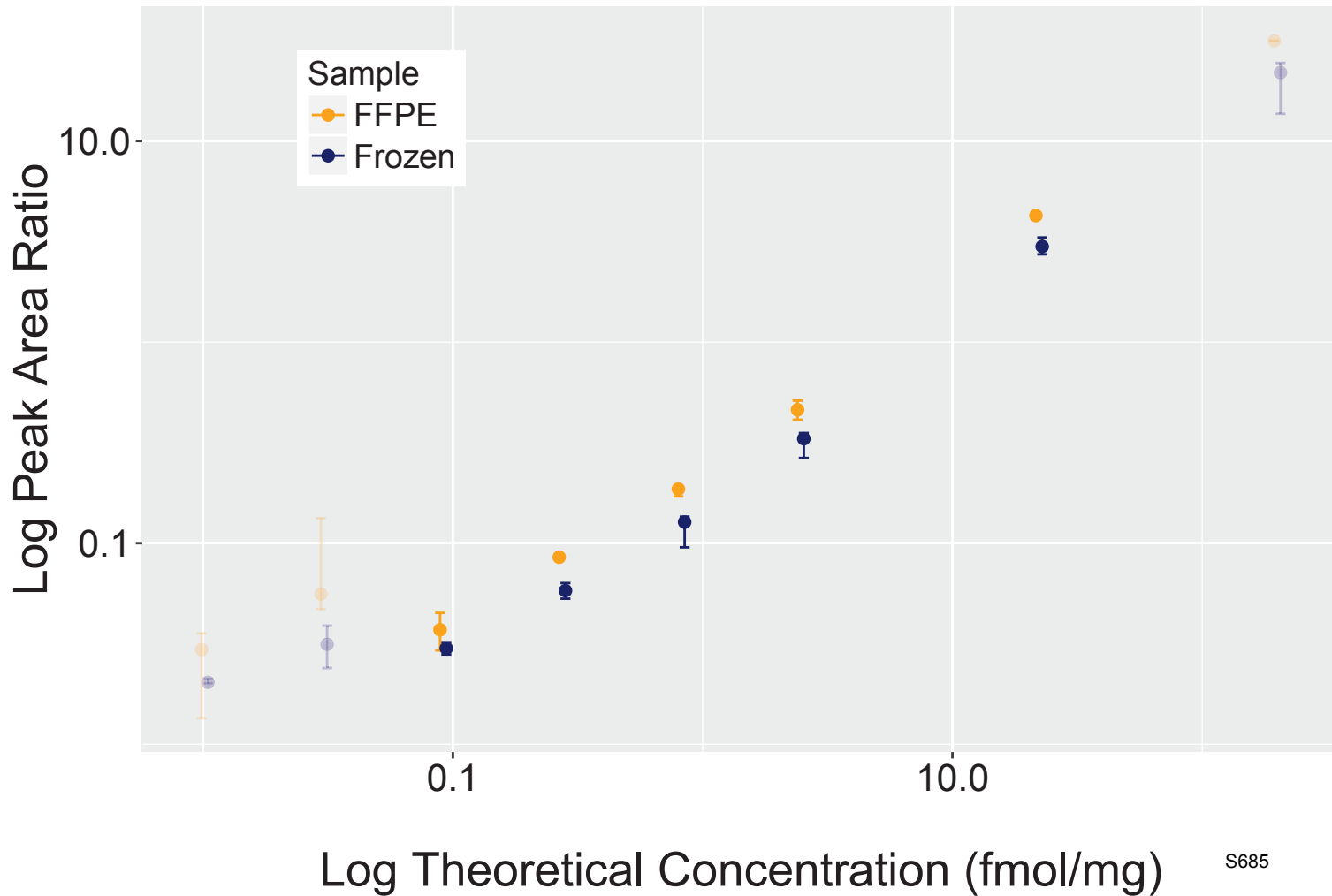
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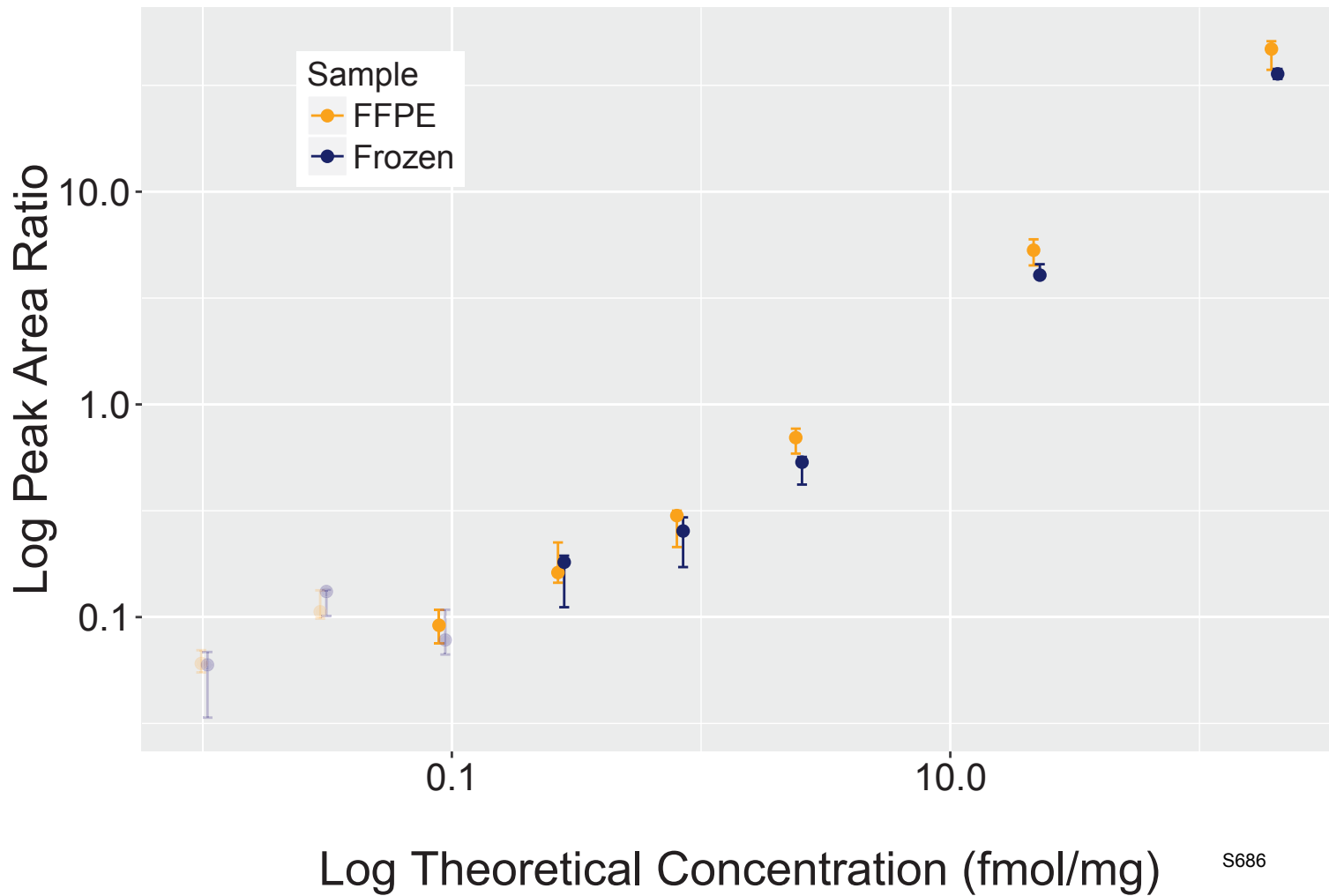
Analyte: DSG3.YTGPTYFALEDQPVK



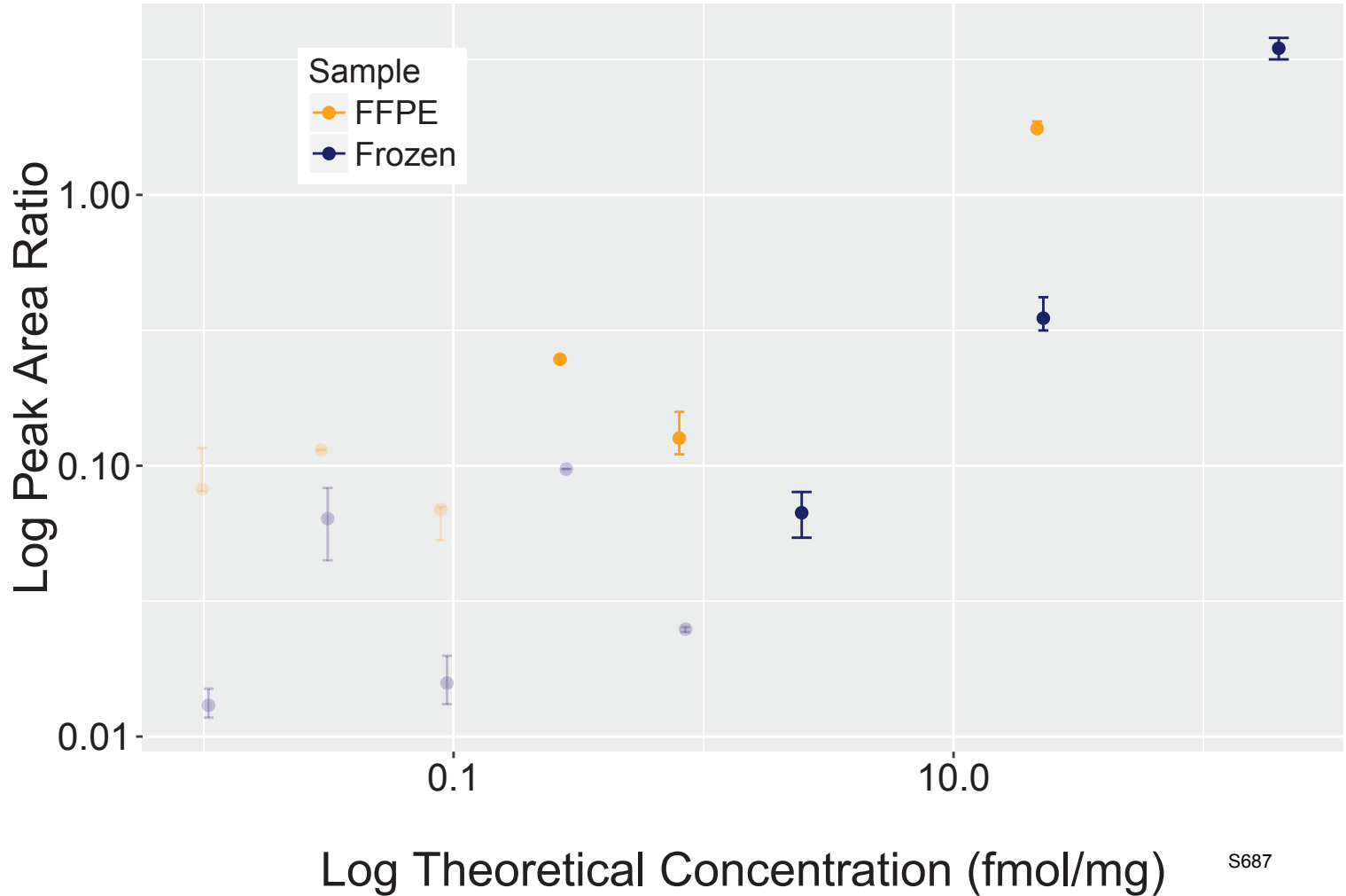
Analyte: TBCB.YTISQEAYDQR



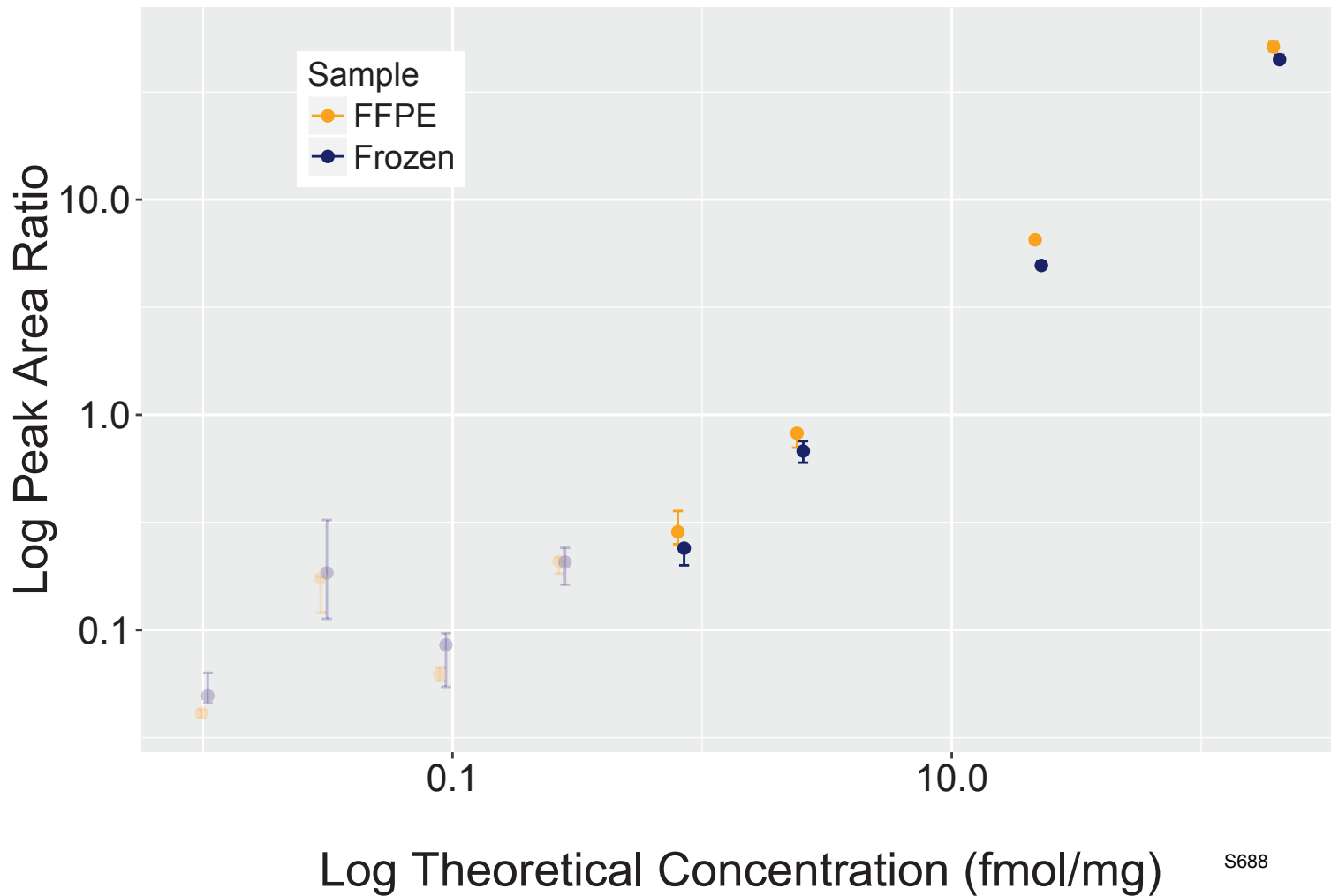
Analyte: MTHFD1.YVVVTGITPTPLGEGK



Analyte: PEBP1.YVWLVEQDRPLK



Analyte: AIP.YYDAIAC[+57]LK



Analyte: PDCD6IP.YYDQIC[+57]SIEPK

